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METALS EFFECT ON FISH TISSUES
I. EFFECTS OF CHRONIC MERCURY AND SELENIUM TREATMENT ON YOUNG TILAPIA TISSUE ENZYMES AND LIPID PEROXIDATION

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Abstract

Chronic treatment was conducted with different concentrations of mercury chloride ($HgCl_2$) and sodium selenite (Na_2SeO_3) alone and an in combination. Effect of chronic treatment was studied on some metallo-enzyme and lipid peroxidation of liver and muscle tissues.

Both metals showed a strong concentration-dependent toxic effect on enzyme activities.

Key words: Mercury chloride and sodium selenite intoxication, tilapia hybrid, enzymes activity changes, lipid peroxidation.

Introduction

Numerous studies deal with the characteristic effect of Hg^{2+} and Se on vertebrates. In earlier works where the effect of $CuSO_4$ respectively, were studied in fishes it was demonstrated that the compounds are affected by the formation of "oxidative stress" in fish tissues. Upon treatment with $ZnSO_4$ oxidative stress similar but slighter is induced (RADY et al., 1988).

It was demonstrated that Hg^{2+} salts induce oxidative damage (state of oxidative stress) first of all in the kidney tissues of rat by dismutating superoxide (O_2^-) anion which resulted in H_2O_2 formation.

Thereby accumulation of H_2O_2 is the cause of oxidative damage. Oxidative

stress presumably decreases the amount of reduced glutathion (GSH) in the tissues, while increases lipid perocidation (LP) (LUND et al., 1991, MILLER et al., 1991). It is known that Se protects the organisms from Hg^{2+} intoxication and is a good anti-oxidant. Therefore the aims of present examination are the study of different concenterations of Se and Hg^{2+} salts alone, and in combination, as supplementation of the artificial diet on the metabolic enzymes and lipid peroxidation on tilapia fingerlings.

Materials and Methods

Experiments with fishes

Tilapia *(Oreochromis niloticus)* fingerlings weighing about 2.3—2.9 g/fish in average, were obtained from Research STation Faculty of Agriculture, Alexandria University, Egypt. They were kept in a glass jar (105 1 capacity) at temperature 28 °C and fed for seven days on a basic diet as adaptation period and the healthy fish were selected for the experiments.

They were then randomly distributed into twenty glass jars filled with tap water which was stored for two days before use. Water was changed every day. Water temperature was thermostatically controlled at 28±1 °C. Diets were prepared by mixing thoroughly the dry ingredients at first and followed wiht oil.

The composition of the basic diet is given in table 1

Table 1. Ingredient on basis dry matter (% d. m.)

Fish meal	20.00
Soybean meal	35.00
Yellow corn	40.00
Bone meal	2.00
Fish oil	2.00
Trace elements	0.70
Vitamin premix	0.30

Before the beginning of the mixing process, stock solution of mercury chloride and sodium selenite were prepared by dissolving 2.187 g of mercury chloride ($HgCl_2$) (solution A) and 1.35599 gm of sodium selenite (Na_2SeO_3) (solution B) in one litre of distilled water. The formulated basic diet consisted of fish meal, yellow corn and bone meal, fish oil, vitamins and minerals and was used as a control diet without the addition of solutions A or B. The other diets tested were prepared by adding 0.25, 0.5 and 1.0 ml of solution A, and 0.5, 1.0 and 1.5 ml of solution B per kg of basic diet, respectively. Mixture of mercury chloride (solution A) and sodium selenite (solution B) were prepared by adding 0.25, 0.5 and 1.0 ml of solution A with 1.5, 1.0 and 0.5 ml of solution B, respectively.

Feeding strategy:

Fish were fed on the experimental diets (two jars for each diet) for 112 days. The feeding rate was as follows: 10% of the total biomass of fish daily for 14 days and 4% of the total biomass of fish for 70 days.

The daily feed ration was divided into two equal portions and give at 9.00 a.m. and 1.00 p.m. The glass jars were cleaned daily to prevent the accumulation of faeces to reduce algae growth.

Fish sampling:

At the end of the experiment five fish from each jar were taken, and their muscle and livers were removed and kept under cold condition for biochemical analysis.

Selenium in fish organs were determined spectrophotometrically by 3,3-diaminobenzidine described by MARCZENKO (1976). Mercury in fish organs was determined by flameless atomic absorption spectrophotometer (perkin Elmer 4305) by the method of CHAPMAN et al. (1961).

Biochemical-measurements:

Alkaline and acid phosphatase (AlPh-ase; EC 3.1.3.1), (AcPh-ase; EC 3.1.3.2) activities were measured according to the method of BERGMEYER (1974) phenol released by enzymatic hydrolysis from phenyl phosphate under defined conditions of time, temperature and pH was measured colorimetrically at 400 nm after using 1N NaOH to stop reaction.

Lactic dehydrogenase activity (LDH; EC 1.1.1.27) was determined colorimetrically using the method reported by ANON (1971) the method depends on reduction of pyruvate by incubation with enzyme in the presence of reduced nicotinamide adenine dinucleotide (NADH). The reaction was stopped by adding dinitrophenyl hydrazine solution, which reacts with the remaining pyruvate forming hydrazone. The colour produced was measured at 510 nm.

Glutathione transferase avtivity (GSH-S-Tr-ase; EC 2.51.18). Enzyme activity is expressed as μmoles of 4-chloro-1,3-dinitrobenzene (CDNB) conjugated/minute/mg protein according to the method of VESSEY et al., (1984), in the presence of reduced glutathione. Glutathione peroxidase activity (GP-ase: EC 1.11.1.9) was measured spectrophotometrically using cumene hydroperoxide and GSH solutions as substrates (CHIU et al., 1976; SEDLAK et al., 1968).

Lipid peroxidation (LP) was measured by the thiobarbituric acid spectral method of PLACER et al. (1966).

Total protein was measured by the Folin-reagent according to the method of LOWRY et al., (1951).

Statistical analysis: The data obtained in the present study were statistically analyzed according to the method of SNEDECOR et al., (1967).

Result

In Fig. 1 it can be seen that AlPh-ase activity of liver homogenate is increasing steadily after selenite treatments, only the highest selenite concentraton (1.5 mg) decrease significantly the enzyme activity. The mercury salt treatment after the acitivation of enzyme, in highest concentration (1.0 mg) totally inhibited the enzyme activity. The two metals together only in the concentrations of 1.5 mg and 0.25 mg respectively inhibited significantly the AlPh-ase activity.

The AcPh-ase activity changes only after the treatment of 1.0 mg of Na-selenite, it activates the enzyme and after the highest mercury treatment (1.0 mg) the activity decrease considerably.

The mild treatment of selenite (1.0 mg) and mercury (0.5 mg) respectively decrease the LDH activity on a concentration dependent manner.

The further effect of metals on the liver enzymes activities and LP summarised in Fig. 2. The selenite treatment activate the GSH-S-Tr-ase as well as GP-ase at lower concentration (0.5 mg) but inhibits them in the highest one similar to mercury. The highest selenite and mercury treatment significantly

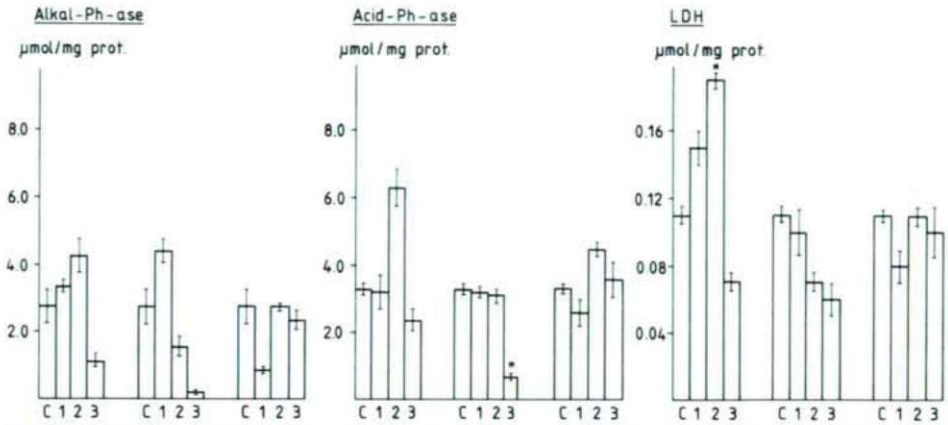

Fig. 1. First series of column shows the changes enzyme activities in liver homogenates if 0.5, 1.0 and 1.5 mg/kg sodium selenite is mixed in the feed (columns No. 1, 2, 3). The second revies of column demonstrates the same with 0.25, 0.50 and 1.0 mg/kg $HgCl_2$. The first colounna of the third series shows the simultaneous effect of 1.5 mg/kg sodium selenite and 0.25 $HgCl_2$; while the second and third coloumnes indicate the simultaneous effect of 1.0 mg: 0.5 mg and 0.5 mg: 1.0 mg selenite and $HgCl_2$, respectively.

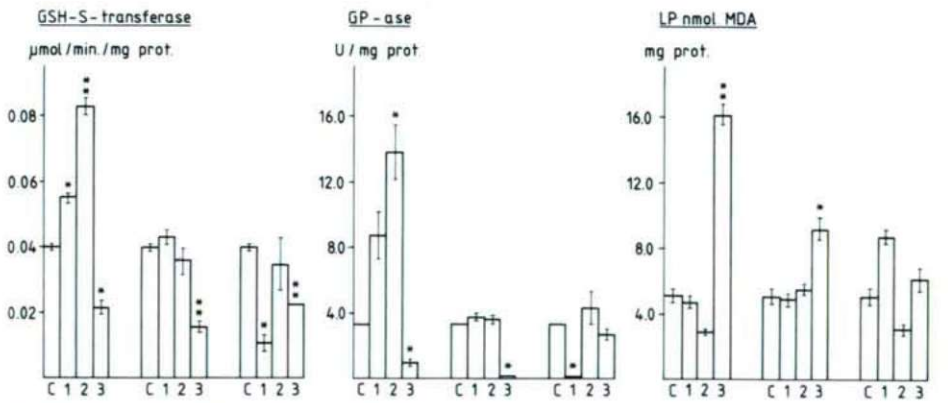

Fig. 2. Effects of metals as listed in Fig. 1 on liver enzyme activities and LP are shown.

increased the LP. Changes in the AlPh-ase, AcPh-ase and LDH activity in muscle are shown (Fig. 3) as wel as the GSH-S-Tr-ase and GP-ase activity and LP (Fig. 4).

The treatments did not influence very much the LDH activity, but the lower concentration of Na- selenite activates the AlPh-ase and AcPh-ase, but the highest selenite and mercury concentrations significantly inhibited the enzymes. Selenite and mercury in their lower concentration significantly activate the GSH dependent enzymes, but in highest concentrations significantly inactivate them (see Fig. 4.). The highest selenite and mercury treatments activated the muscle

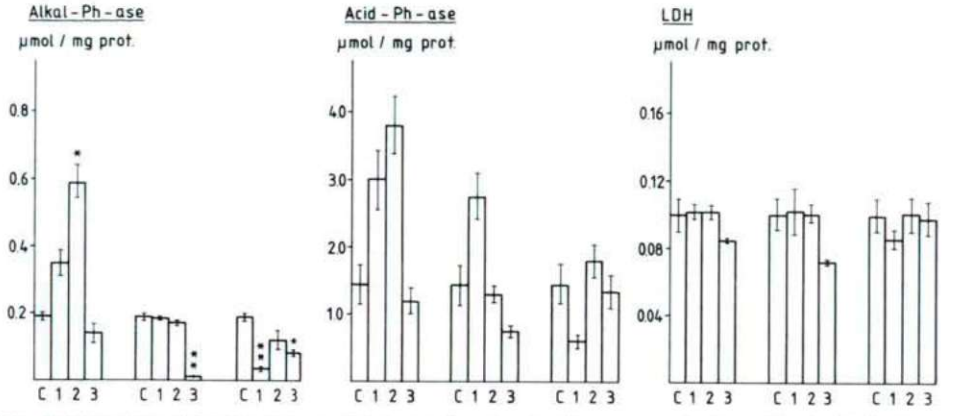

Fig. 3. Response of muscle tissue enzymes upon treatment with metals as indicated in details in Fig. 1.

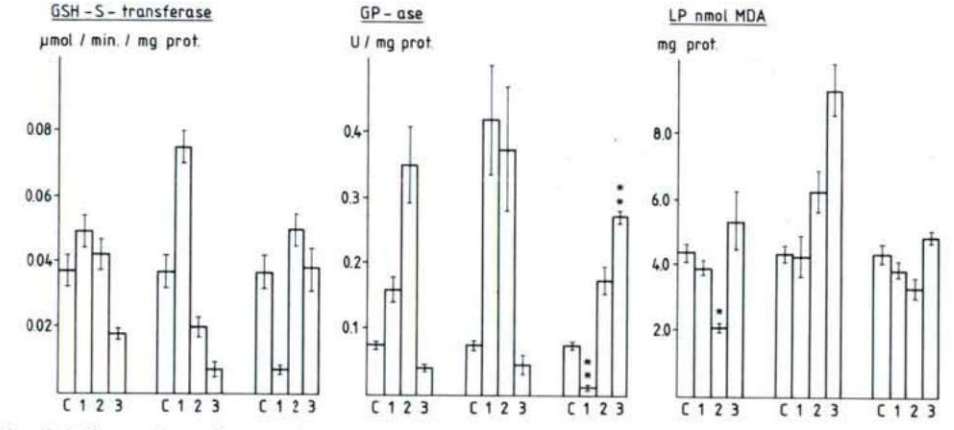

Fig. 4. Effects of metal treatments on muscle tissue enzyme activities and LP vith the same series details as given in Fig. 1.

LP. Table 2. summarises the selenium and mercury measured after the treatments in fish tissues. The Table well demonstrates the concentration dependent increase of metals in the tissues studied upon Se or Hg^{2+} treatments. At simultaneous administration a certain competition could be observed.

Discussion

Se is an enzyme active site specific trace element. Hg^{2+} is a rather toxic pollutant, the amount of which unfortunately is increasing in most of the natural waters. Facts mentioned above made the conducted studies justified. It is also known that both metals, but first of all Se affects the immune system, in such a

Table 2. Concentrations of mercury and selenium of investigated tissues of Tilapia *(Oreochromis niloticus)* fed on different diet contained different levels of mercury and/or selenium.

Treatment	Mercury		Selenium	
	Muscle	Liver	Muscle	Liver
Control	–	–	0.5	1.2
Na_2SO_3 mg/kg feed				
0.5	–	–	0.4	3.8
1.0	–	–	1.5	4.5
1.5	–	–	1.9	5.2
$HgCl_2$ mg/kg feed				
0.25	1.3	1.6	0.5	1.7
0.50	1.3	1.6	0.5	1.7
1.00	1.6	7.9	0.4	1.3
Na_2SO_3:$HgCl_2$ mg/kg feed				
1.5:0.25	0.35	0.23	0.94	5.9
1.0:0.50	0.30	0.14	0.90	2.2
0.5:1.00	0.05	0.30	0.40	3.8

way that Se is an immune activator while Hg^{2+} is immune suppressor element. Immune-toxicity of Hg^{2+} depends upon the Se content of the tissue. Toxic effect of Se manifests, first of all, in its ability to replace S (Sulphur) atom, mostly the active S, e.g. the active S in the Met.

Se can be regarded as the antidote of the toxic effect of Hg (HELLAWELL, 1986 and HEATH, 1987). The dynamic cconcentration dependent of the uptake of the two metals tested with joint treatment in muscle and liver of fish is well illustrated by data in Table 2.

From the studies it appears that Se in the highest concentration used (1.5 mg/kg) is rather toxic for enzymes. It decreases the activities of all other enzymes while increases LP in both liver and muscle homogenates. Cyto-, but first of all nephrotoxic characteristics of Hg are well-known (LUND et al., 1991). Therefore, its tissue enzyme inhibiting and LP enhancing characteristics is not surprising.

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METALS EFFECT ON FISH TISSUES
II. THE EFFECT OF CHRONIC ZINC AND CADMIUM TREATMENT ON YOUNG TILAPIA TISSUE ENZYMES AND LIPID PEROXIDATION

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Abstract

Metals effect on liver and muscle tissues of young Tilapia *(Oreochromis niloticus)* were studied under separate and simultaneous effects of $ZnCl_2$ and $CdCl_2$ during 120 days, with special regards to the respective ion containing active site of the enzymes. Besides the enzymes, changes in lipid peroxidation value in both tissues were also studied.

Key words: Young Tilapia, chronic treatment, zinc chloride and cadmium chloride, tissue enzyme activity, lipid peroxidation.

Introduction

Due to industrial development and the extensive use of chemicals in agriculture, heavy metals are widely distributed in aquatic systems and can affect fish populations, reducing their growth, reproduction and causing significant mortality (VINIKOUR et al., 1980). Similarities in chemical reactivity of cadmium (Cd) and zinc (Zn) lead to similar metabolic pathways in biological systems. Whereas, Zn is an important essential element, acting as co-factor for many enzymes, necessary for DNA synthesis and others. Cd is best known for its toxicity and metabolic antagonism of Zn and other essential elements. Anemia, bone demineralizaton and kidney damage are principal adverse effects of Cd ingested in moderate amount, its higher levels can lead to death (VALLEE et al., 1972). In general Cd can inhibit several key enzymes metabolic processes and it can reduce their ability in osmoregulation. It has been suggested that the environmental contamination of Cd found even in low concentration in the nature, interferes

with the basal metabolism of birds and mammals (SUZUKI et al. 1990a, b, c; MENENDER-BOTET et al. 1991). The aim of the present work was to study the effects of different concentrations of cadmium and zinc (both used in plant protection) on the activity of metabolic enzymes, protein values and level of the lipid peroxidation of tilapia *(Oreochromis niloticus)*.

Materials and Methods

Fish: Two separate experiments were carried on Tilapia *(Oreochromis niloticus)* fingerlings weighing about 4—5 g/fish in average, obtained from the Marjout Fish Farming Company, Alexandria Governorate, Egypt. They were kept in glass jars (105 litre capacity) at temperature of 28 °C and fed for seven days on basic diet. At the start of the experiment healthy fish were weighed and distributed in the experimental jars. Fourteen jars of 105 litres capacity each were used in the experiment and filled with tap water stored for two days before use. Water was changed every three days (about third part changed every day). Water temperature was thermostatically controlled at 28 °C±1 °C.

The formulated basic diet consisted of fish meal, soybean meal, yellow corn and bone meal, fish oil, vitamins and minerals, and was used as control diet RADY et al., (1992) without the addition of stock sol. of A or B.

Experimental diets: Stock solutions of trace metals: cadmium chloride ($CdCl_2$ and zinc chloride ($Zn\ Cl_2$) of 100 ppm concentrations were prepared. They were prepared by dissolving 0.203 g of $CdCl_2$ hydrate with 5 ml of conc. HNO_3 in 1 litre of distilled waater (stock solution A) and one gram of $ZnCl_2$ and 20 ml conc. HCl in one litre of distilled water (stock solution B) suggested by CHAPMAN et al (1961).

The experimental diets were prepared by the addition of 5, 10 and 15 ml from stock solution A per kg of basic diet to give diet 5, 10 and 15 ppm of cadmium, respectively, and 10, 20 and 30 ml of stock solution B added 1 kg of basic diet to give diets of 10, 20 and 30 ppm of zinc concentration. Mixture of $CdCl_2$ (solution A) and $ZnCl_2$ (solution B) were prepared by adding 5,10 and 15 ml of solution A and 30, 20 and 10 ml of solution B respectively.

Feeding Strategy: Fish in every jar were fed on experimental diet (two jars) for each diet at a rate of 8 per cent on the basis of live body weight, first week, then feeding level was reduced to 6 per cent from the third week on, and to 4 per cent from the 7th week. (Daily feed was divided into 100 equal portions and offered for each jar and adjusted every 7-day intervals to the fresh body weight.) The glass jars were cleaned daily to prevent accumulation of faeces. Specimens of different treatments were collected and taken alive to the laboratory in aerated plastic bags.

The apparent examination showed that all fishes were somatically healthy and parasite-free.

Fish sampling: At the end of the experiments five fish from each jar were taken sacrificed by vertebral rupture, then liver and parts from the muscle tissues were rapidly removed. A part of each tissue were weighted and homogenized with 0,64 per cent sodium chloride for biochemical analysis.

Biochemical assays: Total protein was determined by the method of LOWRY et al. (1951), for preparation of calibration curve bovine serum albumin was used.

Lipid peroxidation (LP): Malondialdehyde (MDA) was used as an indicator for lipid peroxides. It was determined by the method described by PLACER et al (1966). Calibration curve was prepared by using malondialdehyde diethyl acetate (Merck, Germany).

Alkaline and acid phosphatase (AlPh-as; EC 3.1.3.1) (AcPh-ase; EC 3.1.3.1) activities were measured activities by the method of BERGMEYER (1974). Phenol released through enzyme hydrolysis

from phenyl phosphate was determined under defined conditions, colorimetrically at 400 nm. Time and pH were also measured.

Lactate dehydrogenase (LDH; EC 1.1.1.27) activity was measured also colorimetrically by the method of ANON (1971). The method is based on reduction of pyruvate by incubation with the enzyme in the presence of NADH. The reaction was arrested by adding dinitrophenyl hydrazine to the solution which reacts wiht the remaining pyruvate, the colour produced was measured at 510 nm.

Glutathione S-transferase (GSH-S-Tr-ase; EC 2.51.18) enzyme activity expressed as μmol of 4-chloro-1,3 dinitrobenzene (CDNB) conjugated /minute/ mg protein according to the method of VESSEY et al. (1984).

Results

In Fig. 1 it can be seen that AlPh-ase activity of liver tissues is increasing steadily after the effect of $ZnCl_2$, while it is gradually decreasing affected by $CdCl_2$, both in the function of concentration. Combination of the two metals, however, do not show any characteristic effect.

AcPh-ase activities are elevated by increased $ZnCl_2$ concentrations, while activating effect of $CdCl_2$ is decreasing along with its concentrations increases. The same characteristic is demonstrated by the simultaneous effects of the two metals. etc. of $CdCl_2$ is demonstrated in Figure 2, which is prevailing, though to a lesser extent upon the treatment with the two metals.

Effect of $ZnCl_2$ and $CdCl_2$, respectively and in combination on GSH-S-Tr-ase activity can also be seen in Fig. 2. Here, Zn has an inhibitory effect, as well as that of the higher concentrations of $CdCl_2$.

Liver parenchyma LP is decreased by $ZnCl_2$, while increased by $CdCl_2$ in the function of the concentration of the latter.

Changes in the activities of the same enzymes in muscle tissues are shown in Figs 3 and 4. Here, striking are the low enzyme activities of muscle-tissues and that all enzyme activities were increased at low- while inhibited at higher concentration of $CdCl_2$.

No noticeable is the LP increasing effect of the highest concentration of $CdCl_2$ in muscle tissues.

The results in Table 1 showed that most of Cd^{2+} an Zn more accumulated mainly, in the liver. Increasing the level of Cd^{2+} and Zn in the diet significantly increased its accumulation in liver and muscle. Zn supplementation greatly reduced Cd^{2+} in the tissues of tilapia.

Discussion

Cadmium occurs widely in the nature in close association with Zn. Cadmium as a human pollutant (Steel industry, waste incineration, volcanic action, Zn production etc...) continuously added to soil, water and air. The main feature of Cd^{2+} toxicity are (i) accumulation in soft tissues (liver and kidney) (ii) inter

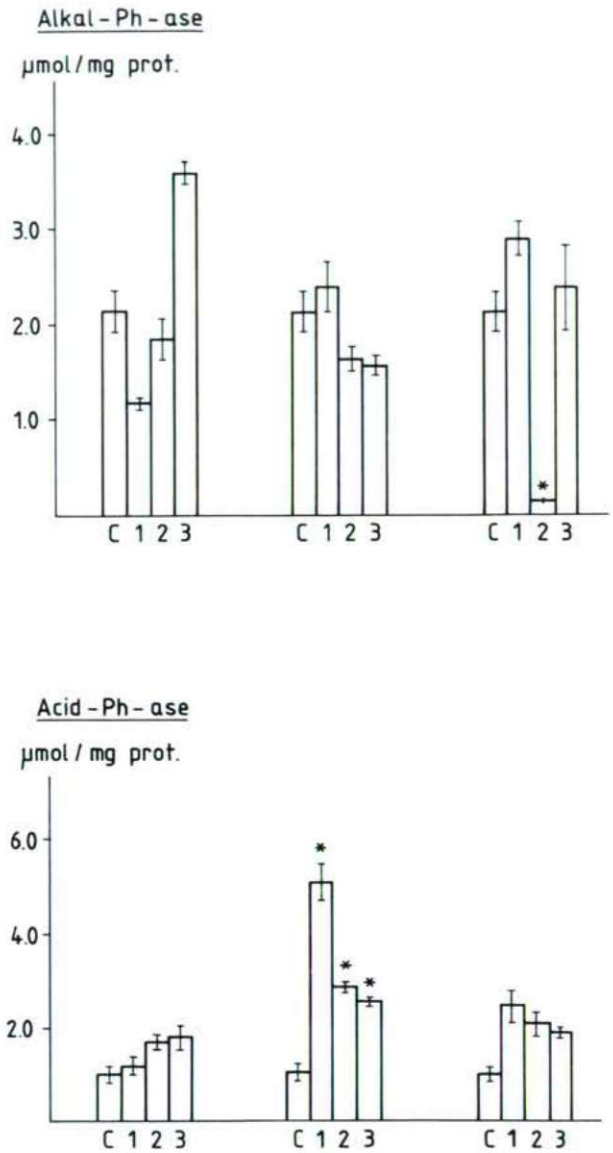

Fig. 1. Effects of metal treatments on liver tissue enzyme activities, first row of columns demonstrate the effect of 10, 20 and 30 ppm $ZnCl_2$ on AlPh-ase and AcPh-ase activities in tilapia liver compared with the control. The second row of columns shows the influence of $CdCl_2$ of 5, 10 and 15 ppm, respectively, on the enzymes. The third row of columns illustrate the simultaneous effect of $ZnCl_2$ and $CdCl_2$ (1 = 30 ppm $ZnCl_2$+5 ppm $CdCl_2$; 2 = 20 ppm $ZnCl_2$+10 ppm $CdCl_2$; 3 = 10 ppm $ZnCl_2$+15 ppm $CdCl_2$; 3 = 10 ppm $ZnCl_2$+15 ppm $CdCl_2$) treatment on enzyme activities.

*and** = p 0.01 and P 0.001 significances, respectively.

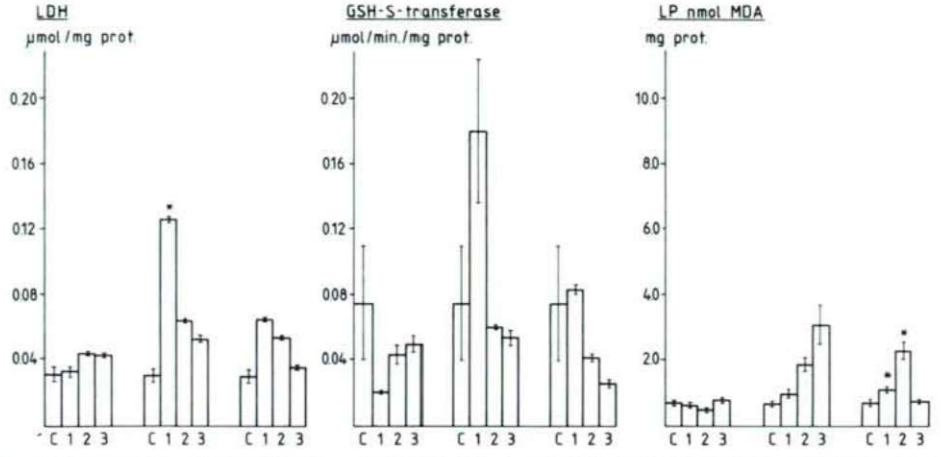

Fig. 2. Sums up the effects of treatments listed in Fig. 1 on LDH and GSH-S-Tr-ase activities and on LP in liver homogenates.

action with other divalent cations (mainly with Zn) (iii) Long half-live time (iv) Very slow elimination. Its metabolism and transport is bound to metallothionein.

As to follow up our eaarlier investigatins with carp (RADY et al. 1988) in the present work it was studied how $ZnCl_2$ and $CdCl_2$, respectively, at three different concentrations, and in combination at various defined concentrations, affect liver and muscle biochemical parameters of young *Tilapia*.

It could be observed that upon the treatment with the two metals, either

Table 1. The distribution of cadmium and zinc in liver and muscle of tilapia *(Orochromis niloticus)* μg/gm w. t.

Treatment	Cadmium		Zinc	
	Muscle	Liver	Muscle	Liver
Control	–	–	0.260	2.43
$CdCl_2$ mg/kg feed				
5	0.245	13.28	0.245	2.320
10	0.265	14.18	0.135	1.820
15	0.318	14.61	0.125	1.500
$ZnCl_2$ mg/kg feed				
10	–	–	0.365	6.070
20	–	–	0.790	7.120
30	–	–	1.180	9.082
$CdCl_2$:$ZnCl_2$ mg/kg feed				
5:30	0.225	10.17	1.250	6.370
10:20	0.240	12.70	0.813	6.330
15:10	0.312	13.57	0.409	5.250

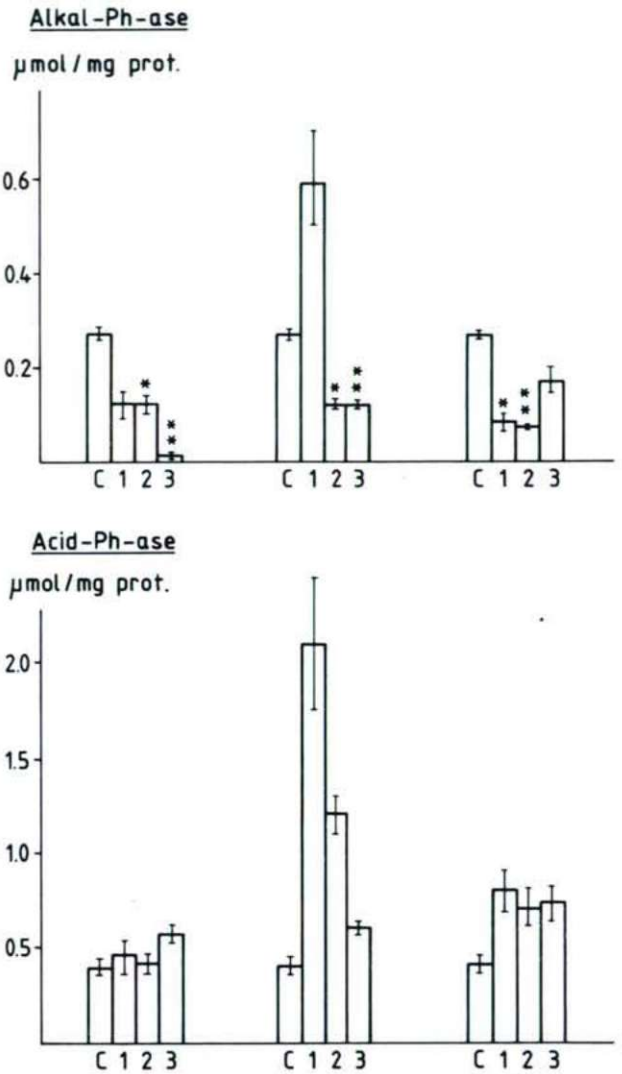

Fig. 3. Effects of treatments detailed in Figure 1 on muscle tissue phosphatases activities.

separately or together, the effect of $CdCl_2$ dominated, which is in line with earlier papers reporting that Cd ion is able to ousting Zn from several important loci of its effect (SUZUKI et al., 1990a; HELLAWELL, 1986 and HEATH, 1987). In additin to lead to significantly enzyme inhibition of white muscle, due to its accumulation increase oxidation of molecular oxygen to superoxide radical, this reaction would act as source of H_2O_2, which inicitiates polymerisation of specific membrane protein.

The present series of investigations might be important from the fact that the enzymes studied contain Zn atom in their active centres.

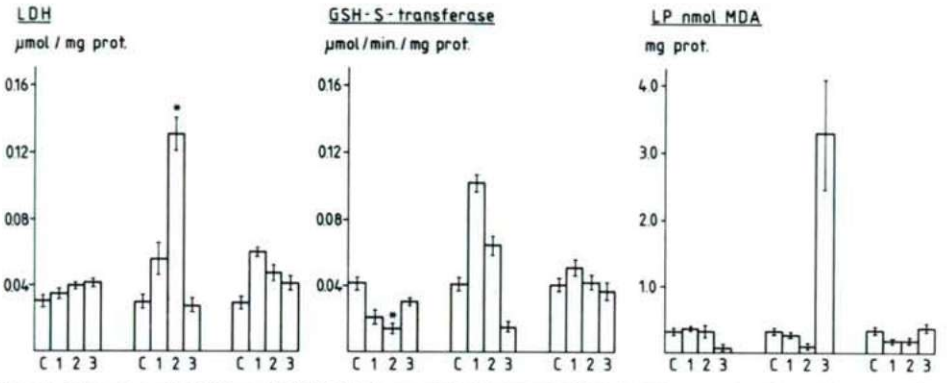

Fig. 4. Changes of LDH and GSH-S-Tr-ase activities and LP values in muscle tissue homogenates upon separate and simultaneous treatments with Zn and Cd.

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THE EFFECT OF VITAMIN E AND SELENIUM OVERLOAD ON RATS ANTIOXIDANT ENZYMES

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Abstract

1. Rats were treated with two concentrations of vitamin E and sodium-selenite and antioxidant enzyme activities and lipid peroxidation, were determined. The following antioxidant enzymes were studied superoxide dismutase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase in serum and liver homogenates.

2. In general the two known antioxidants in low concentration can increase the activities of antioxidant enzymes studied and inhibited lipid peroxidation.

3. On the other hand, the treatment with higher concentrations of the antioxidants changed their original effects and become prooxidants.

Key words: Vit. E and sodium selenite treatments, rats, serum and liver, antioxidant enzyme activities, lipid peroxidation.

Introduction

Lipid peroxidation is a toxic process in biological system. One thoroughly studied agent of peroxidation is the superoxide free radical (O_2^-) which can be generated in several metabolic and enzyme catalyzed reaction and has been found to have detrimental effects on cells and cell constituents (TAPPEL, 1973; CSALLANY et al., 1992). One of the principal biological defense against lipid peroxidatin is α-tocopherol (vitamin E) which is located in biomemberanes and has the capacity to scavenge O_2^-, H_2O_2, HO·, $^1O_2$ and lipid free radicals in vitro. (FUKUZAWA et al., 1985).

Treatment with selenium and vitamin E, so-called antioxidant treatment is well known for elimination of harmful effect of free radicals in the selenium metabolism, to the oxidation of the selenium as reducing equivalents reduced glutathione (GSH) and NADPH are necessary. The reduction of selenite to hydrogen selenite is catalysed by glutathione reductase via the intermediary selenopersulphide step (THOMPSON et al., 1991).

The above statement means that these treatments delay atherosclerotic

processes, aging and in several cases are applied in antitumour therapy (CHOW, 1991; STADTMAN, 1990).

In the present experiments we studied how Se and vitamin E overloading influences antioxidant systems in rats kept on normal diet.

Materials and Methods

Adult Wistar rats of the same age and about the same weight (200—250 g) kept under identical conditions, were used for the experiments. They were randomly divided into five groups. Ten rats were in each group. Water and food was given ad libitum.

The first group which was considered as control, were injected with 0.2 ml saline solution three daily for 24 days and 0.2 ml cotton seed oil once a day. The second and third groups were injected subsequently with 20 mg and 100 mg of vitamin E, (DL- alfa- tocopherol acetate, Sigma-Chemical Co., U.S.A.), respectively, dissolved in 0.2 ml cotton seed oil once a day for 24 days. Other two groups were injected subcutaneously with 0.25 mg and 1.5 mg sodium selenite (Na_2SeO_3), (Sigma-Chemical Co, U.S.A.) respectively, also three daily for 24 days.

Sample collection and measurements: After the above mentioned treatments the rats were sacrificed by decapitation and the blood were collected and the serum were separated. For biochemical analysis only the liver was used after the homogenization of 1 g wet weight in 9 ml physiological saline solution.

In measurement of superoxide dismutase (SOD, EC 1.15.1.1) the weighed liver tissue was homogenized in 0.05 M K_2HPO_4 solution (pH 7.8). Enzyme activity was determined on the basis of inhibition, of epinephrine-adrenochrome autocatalitic transformation (MISRA et al., 1972; MATKOVICS et al., 1977.)

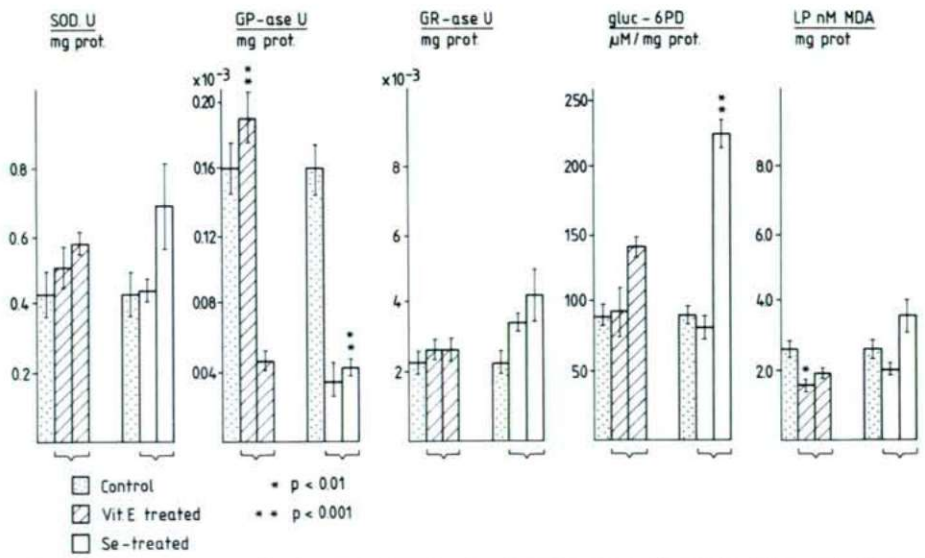

Fig. 1. Rat serum enzyme activities and lipid peroxidation of control, vitamin E and selenium treated animals. (the enzyme activities and LP are given in/mg protein values)

Glutathione peroxidase (GP-ase, EC 1.11.1.9) activity was determined with a chemical method using cumene hydroperoxide as co-substrate. Enzyme quantity was regarded 1 enzyme unit (IU) which transformed 1 micro mol substrate in 1 minute (CHIU et al., 1976; SEDLAK et al., 1968; MATKOVICS et al., 1988).

Glutathione reductase (GR-ase, EC 1.6.4.2) activity was measured with the method described by BERGMEYER et al. (1983) using NADPH (7.8). Unit is the amount of enzyme using 1 micromol NADPH in one minute.

Glucose-6 phosphate dehydrogenase (Gluc-6PD, EC 1.1.1.49) activity was measured with the method described by BERGMEYER et al. (1983) with using NADP-sodium salt as cosubstrate, 1 Unit was the enzyme quantity which could reduce 1 micromol NADP+ in 1 minute at 30 °C.

Lipid peroxidation (LP): Malondialdehyde (MDA) was used as an indicator for lipid peroxides. It was determined by the method described by PLACER et al. (1966). Calibration curve was prepared by using malondialdehyde diethyl acetate (Merck, Germany).

Protein content: Was measured by the method of LOWRY et al. (1951) using Folin-phenol reagent. Calibration curve was prepared with human serum albumin.

Measured data are the means of triplicate from 3 parallels each, which did not show higher deviation than 5% among them.

The results were statistically evaluated with Student's t—test and the correlation coefficients were also calculated.

Results

Results are demonstrated in two figures. Activities of SOD, GP—ase, GR—ase, Gluc—(PD and changes in LP in serum are shown in Fig. 1, while those measured in liver are in Fig. 2. compared to the control values.
The following observation can be made in Fig. 1.
(i) Activity of serum SOD shows a moderate increase upon both Vitamin E and sodium selenite treatment.
(ii) Interestingly, treatment with lower dose of vitamin E induced moderate increase in enzyme activity in case of GP-ase while, treatments with vitamin E in higher concentration showed a significant decrease.
(iii) GR—ase activity was increased by both substances tested, the increase though was more significant upon sodium-selenite treatment.
(iv) Increase of Glu-GPD was significant upon vitamin E as well as sodium selenite in higher concentration.
(v) LP value was decreased by vitamin E and increased by sodium selenite. Variation in the order of magnitude can be observed in Fig. 2, because the activities were measured in liver homogenates.
(i) Total SOD activity increased in the function of antioxidant concentrations. Significant increase in total-SOD activity was induced by treatments with higher amount sodium selenite.
(ii) Low concentration of the substances increased, while higher concentrations decreased GP-ase activity.
(iii) GR-ase activity significantly increased upon the treatments with both substances.

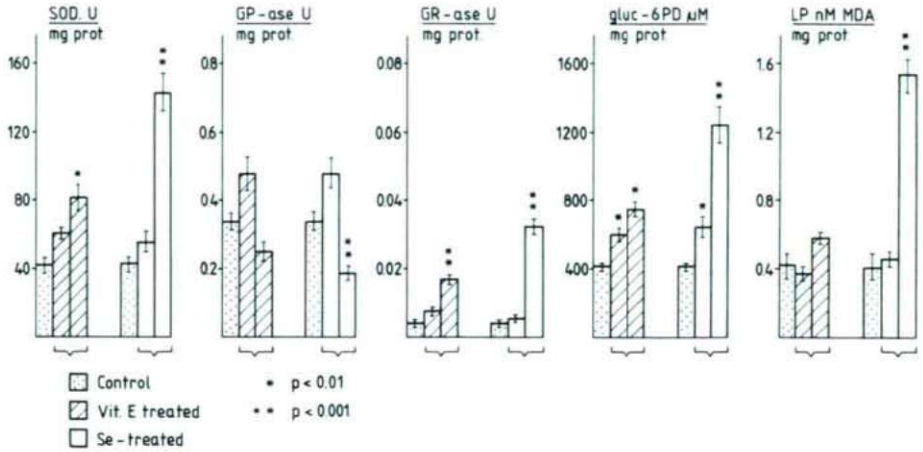

Fig. 2. Enzyme activities and lipid peroxidation of liver homogenisate of the vitamin E and selenium overloaded animals. (the enzyme activities and LP are given in/mg protein values)

(iv) Gluc-6PD activity slightly was increased by both treatment in a concentration dependent manner.
(v) LP value was increased by the higher concentration of vitamin E and sodium selenite overloading.

Discussion

Previous study showed that, vitamin E an effective antioxidant, inhibits lipid peroxidation via donation of a hydrogen atom to a lipid peroxyl radical, thus forming a lipid hydroproxide (LOOH), and reversibly oxidizes vitamin E (CHOW, 1991). This oxidised vitamin E is more rapidly reduced by glutathione in the presence of phospholipid hydroproxide glutathione peroxidase and glutathione (MAIORINO et al., 1989). In the present study we investigated whether low supplementation of vitamin E and selenium increases antioxidant enzyme activities and decreases lipid peroxidation. It has been shown that low levels of vitamin E and selenium can be maintained by glutathione in reduced form (LEEDLE et al., 1990; THOMPSON et al., 1991).

The results of present study are in agreement with HU et al. (1990) obtained from rats on vitamin E diet showed that supplementation of high concentration of vitamin E or selenium lead to significant decrease of glutathione peroxidase and significant increase in lipid peroxidation. It is possible that the level of glutathione in the cells used as cofactor for scavenging lipid peroxide and hydroperoxide, was not enough to reduce of reversibly oxidized vitamin E which resulted in its accumulation in cells (FUKUZAWA et al., 1985).

It can be concluded that vitamin E and selenium, in low concentration

increase the activity of antioxidant enzymes and decrease lipid peroxidation in the liver and serum, while high concentration of both antioxidant can turn over in the cells in oxidised form and cause alteration in the membrane proteins, which decrease the glutathione level. Due to the low level of GSH in the cell the vitamin E becomes prooxidant.

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COMPARATIVE STUDY ON FISH FARMING IN EGYPT

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Abstract

The present paper aims to study the status of fish pond farming in Egypt. Four different fish farms around Alexandria with different sizes (4.2, 21, 16.38, and 617.39 hectares, respectively) and water sources (fresh and brackish) were selected to collect the information of the present investigations. The results showed the following:

1. Water quality criteria showed that temperature ranged between 14.6 °C to 29.3 °C, pH 7.6–8.9, dissolved oxygen 3.5–10.5 mg/l, and salinity 17.8–37.6 mg/l. The concentration of dissolved oxygen was related with seasonality. In the integrated fish farms with ducks, water quality was greatly affected with time of rearing ducks on the pond.
2. Growth performance of silver carp was higher than common carp or tilapia hybrid.
3. Biochemical and chemical analysis of the fish muscles showed that tilapia spp. and *Latus niloticus* contain higher protein amount, while the lower protein was found in common carp *(Cyprinus carpio L.)* flesh of different fish species grew in ponds.

Key words: Fish farming, Egypt, environment influence, carp and tilapia hybrid.
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Introduction

Fish plays an important role in providing food and employment. It can compensate for the shortage of animal protein speially during childhood. In Egypt the practice of fish farming is very old, a base relief found on an ancient Egyptian tomb shows tilapia being harvested of an artificial pond, presumably a drainable one. This base relief is an evidence that fish farming was already practised in Egypt about 2.500 B. C. The Egyptian fisheries can be grouped into three broad groups including marine, lake and inland fisheries, with a water area of about 2.5 million hectare (FAO, 1985). However, the annual rate of fish consumption per capita (4 kg) in Egypt is comparatively low when compared with that of Japan (36 kg), Ceylon (20.3 kg) and Kuwait (18.4 kg) (FAO, 1981). The annual catch of fish production is about 150 thousand tons and the amount of imported fish to Egypt reached 100 thousand tons (FAO, 1985). A great attention is now being given to fish farming for raising fish under controlled

conditions and for development of the inland fishery resources in Egypt. The selected fish species for cultivation should have a rapid growth rate, successfully reproduce accept natural and cheap artificial food, proved satisfactory to be consumer, support high population density and be resistant to disease. So the scientific management aims to produce a low- coast fish by utilization of natural food specially organic and inorganic fertilizers in fish ponds (WOYNAROVICH et al., 1963; WOYNAROVICH, 1988; RADY, 1992) obtained an increase in growth rate of fish 1 kg/9—13 kg fertilizer.

The aim of the present work is to asses the following points:

1. The effect of integrated duck-fish farming with ducks on water quality.
2. Growth of some culture fish species in ponds.
3. Chemical composition of fish flesh.

Material and Methods

The data of this work were based on the registered records and field observations experimental information from four fish pond farms in Alexandria governorate.

The selected fish pond farms were:

1. Experimental fish farm of Alexandria University in Abis, Alexandria (Alex.).
2. Fish farm Alexandria governorate in Al-Nozha, hydrodrome drain, Alex. (Farm 400).
3. Fish farm El-Nozha, hydrodrome Lake Alex.
4. Alexandria Governorate fish farm in Maruit (306).

The total area of the four fish farms was 4.2, 21, 617.39 and 16.38 hectares, respectively, with a pond area in average of 0.42, 7.35, 839.98 and 5.04 hectares, respectively. The average depth of water in the ponds were 1.0, 3.5, 2.0 and 1.25 meter in respectively. Fresh water (salinity 250 ppm) is used in supplying feeding of 1st and and 3rd farm, however, farms 2nd and 4th were from brackish water source (salinity 3000 ppm). Water quality data were obtained from the last three farms. In the

Table 1. Growth performance of some cultivator fish species ponds

Item	Tilapia hybrid	Common carp	Silver carp
Initial length (cm)	3.0	7.8	3.0
Initial weight (g)	3.4	6.8	1.5
Final length (cm)	16.9±0.9	24.5±1.62	22.5±1.8
Final weight (g)	166.0±28.96	484.0±151.6	315.1±79.4
Experimental period days	329	329	329
Gain (g)	162.6	478.0	313.6
Average daily gain (g/day)	0.49	1.45	0.95
Specific growth rate (SGR %)	1.17	1.30	1.61
Condition factor	3.4	3.3	2.8

The values are expressed as means of ten animals.

2nd and 4th farms ducks were directly reared on the water surface with different times of production. Growth performance of some selected fish species were followed in the 4th farm in the period between 1989 and July 1990. About 1500 fingerlings of tilapia hybrid (Oreochromis niloticus×*O. aureus)*, common carp *(Hypophthalmichthy molitrix)* were rared in the cages 6 m^2 cages (2 m×3 m) with 1 m depth at rate of 10 fish/m^3.

Samples of then fishes were obtained at different times in order to observe the body weight changes.

Fish were collected for chemical analysis from two ponds of experimental fish farm, Faculty of Agriculture, Alexandria University during the harvesting period (October, November, 1990). The fish samples were homogenized, by using and electrical homogenizer (20 to 30 fishes of the same species monthly). For studying the water quality (dissolved oxygen, pH, water temperature, ammonia and nitrate) of experimental ponds monthly at 7.00 a.m., Dissolved oxygen was recorded according to the Winkler's method, nitrate and ammonia described by GOLTERMANN et al., 1978. pH was measured in situ using an Orion Research (USA) digital pH meter against standard solutions. Chemical analysis of fish flesh was carried out according to the methods described by NAUMAN et al., (1976).

Results and Discussion

The data in Table 1 showed the growht performance of three cultivated fish species, tilapia hybrid (*Oreochromis niloticus+O. aureus)* common carp *(Cyprinus carpio* L.) and silver carp *(Hypophthalamichthys molitrix)* through the experimental period of 329 days. The initial weight of fish were 3.4, 6.8 and 1.5 g for the tested speices, respectively. The final weight was 166, 484 and 315 with an average daily gain of 0.49, 1.45 and 0.96 g/day. The specific growth rate (SRG %) of the cultivated fish species were 1.17, 1.30 and 1.61%, respectively. From the obtained results it could be concluded that common carp and silver carp grew faster than tilapia hybrid. The overall average of gain for the three tested species was around 1.0 g/fish/day through the experimental period (about 11 months). Growth performance of silver carp was higher than other tested species this could be explained on the basis of its higher turnover of energy in substance and low excretion rate (VIOLA et al., 1983). The highest value of well-being factor was recorded in tilapia followed by common carp and the lowest was silver carp. It was concluded that increase of plant protein and indigestible polysacharid in the diet of tilapia and carp resulted in non improvement of its growth performance (VIOLA et al., 1977). On the other hand, it was found that increased animal protein in the diet resulted in an increase of the body weight of fish, length and well-being factor (EL-DAHAR, 1988).

The chemical composition (%) of fish (Table 2) showed that *Tilapia niloticus* spp. contain more protein, (BARLATIN et al., 1979), as well as in common carp have more fat and bighead carp have more minerals (ash). Energy content (Kj/g) was higher in common carp as bighead carp, however, other tested species were similar energy content.

To compare the chemical composition (%) of the edible parts of fresh water fishes they contained more crude protein, fat and energy than in marine water.

Table 2. Chemical composition (%) of flesh from pond culture as compared with marine fish

Fish species	% on DM basis				
	DM %	Ash	Crude protein	Ether extract	Energy content Kj/g
1-Fresh water fishes					
A-*Tilapia* spp.					
O. nilotica	22.25	4.98	80.59	14.43	20.97
O. galilia	23.58	5.28	83.13	11.59	20.36
O. aurea	25.79	5.32	81.00	13.68	20.76
T. zillii	24.13	5.88	80.79	13.36	20.59
B-Common carp					
Cyprinus carpio	32.24	5.39	72.26	28.35	24.82
C-Chinese carp					
Chenophrynoodon indellus	23.61	7.71	79.17	13.12	20.19
Hypophthalmichthys molitrix	17.09	7.35	78.50	14.15	20.46
Aristichthys nobilis	16.00	6.80	71.10	21.50	22.02
D-*Mugil capito*	3.13	6.85	76.81	16.94	20.01
E-*Clarias lazera*	26.02	8.17	75.64	16.19	20.72
F-*Latus niloticus*	16.39	5.55	83.30	11.15	20.21
G-*Labeo niloticus*	22.58	6.08	78.63	15.29	20.93
Mean of fresh Water fish	21.98 ±3.5	6.29 ±1.07	78.45 ±3.79	15.76 ±4.79	21.00 ±1.31
2-Mean of marine water* Water fish +	26.72 ±1.2	22.13 ±6.38	68.99 ±6.38	9.74 ±2.20	17.17

* Adapted from WASSEF (1985c). The result represents the average of sic pelagic species by pusse-seine, namely: *Sardinella aurita (*Silt-ardine), *Sardina Pilchardus* (pilchard), *Boops boops* (Bugue), *Irachurus mediterranues* (Horse-mackerel), *Scomber Japonicus* (Blue maykerelior scomber) and *Engraulis encresicholus* (Anchovy).

This clearly shows the higher potentially of freshwater fish as a source of nutrients. (see Table 2)

Results in Table (3.) show some water quality criteria in different seasons for water outlet obtained from the 2nd fish farm. There were a great variation in

Table 3. Physical and chemical properties of water in fsh ponds

Item	Summer	Autumn	Winter	Spring	Mean±SE
Temp. °C	29.30	23.00	14.60	20.7	21.90±6.10
pH	8.70	8.90	8.90	9.1	8.90±0.20
O_2 (mg/l)	3.70	10.50	6.20	5.2	7.20±2.80
NH_3 (mg/l)	0.45	0.24	0.19	0.21	0.27±0.10
NO_3 (mg/l)	0.04	0.07	0.05	0.04	0.05±0.01

water temperature between summer and winter. BARLATIN et al., (1979) reported that tilapia is largely restricted to regions within the 20 °C winter isotherms. Outside of this range it is necessary to heated ponds. In Japan, MARUYAMA (1958) obtained 94.6% survival of *Sarotherodon niloticus* at a low temperature. These fish were then able to survive below 14 °—16 °C. It was noted that *S. mossambicus* failed to introduce in Egypt because the fish became inactive at low temperature. Growth stops and fish die if handled at temperature below 15 °C, and against meeting ponds during the winter months. Temperature not only affects the survival and distribution of species, but also on the fish growth rate and reproduction as well as susceptibility to diseases. BISHAI (1975) showed that a range of 17.2 °C to 19.6 °C below which tilapia decrease in growth rate. Temperature differences between spring and winter reached about 15 °C. however, variations in pH values and dissolved oxygen were not too much between the different seasons. Ammonia concentration increased during summer followed by autumn and decreased in spring, however, nitrate concentration increased in autumn followed by winter and decreased in summer and spring. The average pH value in the water pond was 8.9±0.2, however, HUET (1972) recommends a pH 7—8 as a best for fish cultivation, and the maximal acceptable pH level varies with species.

Data in Table (4.) showed that the dissolved oxygen concentration decreased from 5.4 to 3.5 in the long term duck production. This probably caused by continuous consumption of dissolved oxygen through organic matter oxidation as well as increased amount of plankton die-offs (SWINGLE, 1969) and also may be due to accumulation of toxic intermediate components formed and transportation of organic matter has become limiting factor in the process of intensivve fish production in the pond causes the cessation of fish growth (SHILO et al., 1982). The results showed that pH values increased, this was an indication of increasing

Table 4. Effect of long term duck production on water quality of ponds

Water quality criteria	Pond No.			Average±SD
	1	2	3	
A- Without ducks				
Dissolved oxygen	5.40±0.50	5.10±1.40	5.70±1.40	5.40±0.30
pH	7.30±2.00	7.80±0.70	7.20±0.70	7.40±0.26
NH_3 mg/l	0.35±0.01	0.42±0.02	0.27±0.03	0.35±0.06
NO_3 mg/l	0.06±0.02	0.08±0.03	0.04±0.02	0.06±0.02
B- With ducks				
Dissolved oxygen	3.30±1.20	3.80±1.60	3.40±1.50	3.50±0.19
pH	9.30±0.60	8.60±0.60	8.40±0.40	8.80±0.38
NH_3 mg/l	0.60±0.02	0.64±0.03	0.70±0.02	0.65±0.04
NO_3 mg/l	0.37±0.04	0.18±0.05	0.21±0.02	0.25±0.08

1,2,3: The number of ponds had 433, 434 and 471 duck/4200 meter for 217 days.

ammonia concentration in duck manure its levels reaching 0.7 mg/l in both integrated and fish ponds. However the unionized form of ammonia which is the most toxic form to fish constituted no more than 3% in the fish ponds without duck, while it reached 32% in the fish ponds with duck whereas it found that the toxic level of ammonia lies between 0.6–2 mg/l (EIFAC, 1973) for short time exposure. Nitrate content at the integrated ponds is highly variable ranging between 0.18–0.37 mg/l with an average 0.25 mg/l while another ponds contain very little amount of nitrate ranged between 0.04–0.08 mg/l. Ponds must be fertilized regularly in order to maintain rich plankton flora and fauna therefore all of the animal branches could be integrated efficiently with fish production by utilizing the manure for increasing fish production. The most advantageous, however is to integrate duck production with fish farming. Ducks may be raised in the fish ponds themselves, their manure need not be collected, transported and distributed, but it is dropping directly into the water of the fish ponds.

It could be concluded that the effect of long-term duck production on fish pond resulted in decreasing oxygen content and higher pH value, therefore, good and careful management should be followed, in order to know the suitable periods and numbers of ducks should be put on a certain area in order to keep the water quality in good conditions in order to incrase fish production.

From the observations described it could be concluded that:

1. Growth of cultivated fish species in pond is promising.
2. Integrated fish production with duck need more research because the effect of long-term production of ducks on fish ponds are great influence.
3. *Tilapia* spp and *Latus niloticus* contain higher protein content in its flesh than other tested species, respectively.

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ÉTUDES PALYNOLOGIQUES DES COUCHES DU TERTIAIRE INFÉRIEUR DE LA RÉGION PARISIENNE. VII.

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Résumé

Les espèces des genres de forme suivants sont présentées: *Triatriopollenites, Labraferoidaepollenites, Alabroidaepollenites, Momipites, Labrapollis, Zaklinskaiaepollenites, Plicatopollis, Platycaryapollenites.* Description d'un genre de forme nouveaux, et discussion des problèmes de la nomenclature, en particulier chez les pollens myricoides et platycarioïdes.
Mots clés: Palynologie — *Angiospermae* — Tertiaire inférieur — Région Parisienne.

Pollens myricoides

Les espèces du genre de forme *Triatriopollenites* PF. 1953a ont été classées par THOMSON et PFLUG (1953) dans les sections suivantes: *Anuloferoidae* PF. 1953a, *Labraferoidae* PF. 1953a, *Alabroidae* PF.1953a. Le genre de forme *Momipites* WODEHOUSE 1933 emend. par NICHOLS 1973 et par FREDERIKSEN et CHRISTOPHER 1978 renferme partiellement, ou presque globalement, les espèces du genre de forme *Triatriopollenites* PF. 1953a aussi (sauf l'espèce type — *T. rurensis* PF. et TH. 1953). Selon POTONIÉ (1960) chez les pollens à trois atrium les genres suivants peuvent entrer en ligne de compte: *Engelhardtioipollenites* R. POTONIÉ 1951, *Engelhardtioidites* POT., THOMS. et THIERG. 1950, *Momipites* WODEHOUSE 1933, *Myricipites* WODEHOUSE 1933, *Myricaceoipollenites* R. POT. 1951 et *Triatriopollenites* (PFLUG 1952) THOMSON et PFLUG 1953. E. NAGY (1969) a classé le *T. rurensis* PF. et TH. 1953 dans le genre de forme *Myricipites* WODEHOUSE 1953 sans tenir compte des remarques de POTONIÉ (1960, p. 119). Vu les travaux de NICHOLS (1973), NICHOLS et OTT (1978) et FREDERIKSEN et CHRISTOPHER (1978) il nous semble qu'il est fort désirable de préciser encore les caractères du genre de forme *Momipites* WODEHOUSE 1933 emend. NICHOLS 1973, FREDERIKSEN et CHRISTOPHER 1978. La section *Alabroidae* PF. 1953a est à peu près identique à ce genre. Le *Myricipites* WODEH. 1933 est probablement equivalent à la section *Labraferoidae* PF. 1953a, et il faut retenir le genre de forme *Triatriopollenites* pour les pollens de la section *Anuloferoidae* PF. 1953a. Mais pour celà il faut réexaminer le matériel de WODEHOUSE (1933) et discuter à nouveau le problème du *Momipites* WODEHOUSE 1933 emend. NICHOLS 1973, FREDERIKSEN et CHRISTOPHER 1978. Il nous semble, que les amincissements

polaires de l'exine ont une importance taxonomique, de ce point de vue le genre de forme *Paraalnipollenites* HILLS et WALLACE 1969, et le *Maceopolipollenites* LEFFINGWELL 1971 sont à mentionner. Vue les données bibliographiques ces pollens sont beaucoup plus abondants en Amérique du Nord qu'en Eurasie.

Fgen.: *Triatriopollenites* PF. 1953a emend. KDS. et RUSS. 1982

1. *Triatriopollenites roboratus* PF. 1953a (Pl. I, fig. 1—6)
Présence: Thanétien, zone II: Anizy-le-Château, Thanétien, zone III: Rollot 21/6—16; Sparnacien inférieur: Saint-Léger-aux-Bois 21/6—6a, 21/6—3, Sparnacien moyen: Boulogne-la-Grasse 21/6—18, Chavot, Sinceny 21/6—12.
Appartenance botanique probable: *Myricaceae.*

2. *Triatriopollenites sauerae* (GLADK. 1965) KDS. 1974 (Pl. I, fig. 35—38)
Présence: Thanétien, zone II: Anizy-le-Château, Thanétien, zone III: Rollot 21/6—16; Sparnacien, Facies Argiles des Flandres: Templeuve-en-Pévele B_1—25; Cuisien supérieur: Troesnes I—III.
Appartenance botanique probable: *Myricaceae.*

3. *Triatriopollenites minimus* (GLADK. 1965) KDS. 1974 (Pl. II, fig. 9—16)
Présence: Thanétien, zone II: Anizy-le-Château, Thanétien, zone III: Rollot 21/6—16; Sparnacien inférieur: Arpenty B_1—118; Sparnacien moyen: Boulogne-la-Grasse 21/6—18, Guitrancourt B_1—32; Sparnacien supérieur: Sinceny 21/6—7, 8.
Appartenance botanique probable: *Juglandaceae, Engelhardtia.*

4. *Triatriopollenites engelhardtioides* (ROCHE 1973) ROCHE et SCHULER 1976 (Pl. II, fig. 17—20).
Présence: Thanétien, zone III: Rollot 21/6—16; Sparnacien moyen: Sinceny 21/6—12.
Appartenance botanique probable: *Juglandaceae, Engelhardtia.*

Fgen.: *Labraferoidaepollenites* (PF. 1953a) KDS. et RUSS. 1982

1. *Labraferoidaepollenites pseudorurensis* (PF. 1953a) KDS. et RUSS. 1982 (Pl. I, fig. 19—22)
Présence: Sparnacien supérieur: Nointel.
Appartenance botanique probable: *Myricaceae.*

2. *Labraferoidaepollenites conspicuus* (GLADK. 1965) n. comb. (Pl. I, fig. 23—30)
Syn.: 1965. — GLADKOVA, *Myrica conspicua* sp. nov., p. 168, 169 Plate IV, 1.
1974. — KEDVES, *Triatriopollenites conspicuus* (GLADK. 1965) n. comb., p. 46, Plate XVI, fig. 28—30.

Présence: Thanétien, zone II: Anizy-le-Château; Sparnacien moyen: Boulogne-la-Grasse 21/6—18, Chavot; Sparnacien supérieur: Guitrancourt B_1—32.
Appartenance botanique probable: *Myricaceae.*

3. *Labraferoidaepollenites intermedius* (GLADK. 1965) n. comb. (Pl. I, fig. 39—46)
Syn.: 1965. — GLADKOVA, *Myrica intermedia* sp. n., p. 162, — Plate I, fig. 5.
1974, KEDVES, *Triatriopollenites intermedius* (GLADK. 1965) n. comb., p. 47, Plate XVII, fig. 10—12.
Présence: Thanétien, zone II: Anizy-le-Château, Thanétien, zone III: Rollot 21/6—16; Sparnacien moyen: Boulogne-la-Grasse 21/6—18; Sparnacien supérieur: Nointel, Neuilly—46, Sinceny 21/6—9, 10, 11.
Appartenance botanique probable: *Myricaceae.*

4. *Labraferoidaepollenites dilatus* (FAIRCHILD 1966) KDS. et RUSS. 1982 (Pl. II, fig. 1—8)
Note. — NICHOLS (1973) a classé cette espèce de forme dans le genre *Momipites* WODEHOUSE 1933 emend. NICHOLS 1973, FREDERIKSEN et CHRISTOPHER 1978.
Présence: Thanétien, zone II: Anizy-le-Château; Sparnacien inférieur: Saint-Léger-aux-Bois 21/6—6a; Sparnacien moyen: Boulogne-la-Grasse 21/6—18; Sparnacien supérieur: Neuilly—46, Sinceny 21/6—7, 8, 21/6—9, 10, 11; Lutétien supérieur: Paris, Austerlitz.
Appartenance botanique probable: *Juglandaceae* cf. *Engelhardtia.*

Fgen.: *Alabroidaepollenites* (PF. 1953a)
KDS. et RUSS. 1982

1. *Alabroidaepollenites aroboratus* (PF. 1953a) KDS. et RUSS. 1982 (Pl. I, fig. 7—18)
Présence: Thanétien, zone II: Anizy-le-Château, Thanétien, zone III: Rollot 21/6—16; Sparnacien inférieur: Saint-Léger-aux-Bois 21/6—3; Sparnacien supérieur: Guitrancourt B_1—32, Sinceny 21/6—9, 10, 11; Lutétien supérieur: Paris, Austerlitz.
Appartenance botanique probable: *Myricaceae.*

2. *Alabroidaepollenites palaeogenicus* (BOLOTNIKOVA 1975) n. comb. (Pl. I, fig. 31—34)
Syn.: 1975. — BOLOTNIKOVA, *Engelhardtia paleogenica* sp. nov., p. 104, pl. XX, 8, 9.
Note. — Il n'est pas exclu, que cette espèce de forme soit classée plus tard dans le genre de forme *Momipites* WODEHOUSE 1933 emend. NICHOLS 1973, FREDERIKSEN et CHRISTOPHER 1978. C'est le sujet avec les autres espèces décrites par BOLOTNIKOVA (1975) dans le genre *Engelhardtia.*

Présence: Thanétien, zone III: Rollot 21/6—16; Sparnacien supérieur: Guitrancourt B_1—32, Neuilly—46; Facies Argiles des Flandres: Templeuve-en-Pévèle B_1—25; Cuisien supérieur: Troesnes I—III; Lutétien supérieur: Paris, Austerlitz. Appartenance botanique probable: *Juglandaceae, Engelhardtia.*

Note. — Selon nos connaissances actuelles les espèces suivantes peuvent être classées dans les genres de forme mentionnées précédemment:

Triatriopollenites diversus (GLADK. 1965) n. comb.
Syn.: 1965. — GLADKOVA, *Myrica diversa* n. sp., p. 166, 167, pl. III, 3,4, holotype, fig. 3.

Triatriopollenites galiformis (GLADK. 1965) n. comb.
Syn.: 1965. — GLADKOVA, *Myrica galiformis* n. sp., p. 167, 168, pl. III, 5,6, holotype, fig. 5.

Triatriopollenites clementinus (GLADK. 1965) n. comb.
Syn.: 1965. — GLADKOVA, *Comptonia clementina* n. sp., p. 174,175, pl. V, 3,4, holotype, fig. 3.

Triatriopollenites imperfectus (GLADK. 1965) n. comb.
Syn.: 1965. — GLADKOVA, *Comptonia imperfecta* n. sp., p. 178, 179, pl. VIII, 3,4, holotype, fig. 3.

Labraferoidaepollenites tenuis (GLADK. 1965) n. comb.
Syn.: 1965. — GLADKOVA, *Myrica tenuis* n. sp., p. 159—161, pl. I, 1,2, holotype, fig. 1.

Labraferoidaepollenites paradoxus (GLADK. 1965) n. comb.
Syn.: 1965. — GLADKOVA, *Myrica paradoxa* n. sp., p. 163, 164, pl. II, 1—3. holotype, fig. 1.

Labraferoidaepollenites aborigenus (GLADK. 1965) n. comb.
Syn.: 1965. — GLADKOVA, *Comptonia aborigena* n. sp., p. 175, 176, pl. VI, 1—5, holotype, fig. 1.

Labraferoidaepollenites insolitus (GLADK. 1965) n. comb.
Syn.: 1965. — GLADKOVA, *Comptonia insolita* n. sp., p. 181, 182, pl. X, 6.

Labraferoidaepollenites peregriniformis (GLADK. 1965) n. comb.
Syn.: 1965. — GLADKOVA, *Comptonia peregriniformis* n. sp., p. 183, 184, pl. XI, 3,4, holotype, fig. 3.

Alabroidaepollenites compactus (GLADK. 1965) n. comb.
Syn.: 1965. — GLADKOVA, *Comptonia compacta* n. sp., p. 182, 183, pl. XI, 1,2, holotype, fig. 1.

Fgen.: Momipites WODEHOUSE 1933 emend. NICHOLS 1973, FREDERIKSEN et CHRISTOPHER 1978

Note. — POTTER (1976) a soutenu la thèse de l'affinité des *Juglandaceae (Alfaroa, Engelhardtia, Oreomunnea)* avec ces pollens.

1. *Momipites kedvesii* (GRUAS-CAVAGNETTO 1967) FREDERIKSEN et CHRISTOPHER 1978 (Pl. II, fig. 21, 22)

Présence: Sparnacien supérieur: Sinceny 21/6—7, 8, 21/6—9, 10, 11; Cuisien supérieur: Troesnes I—III, Fosses: I—III.

Fgen.: Labrapollis W. Kr. 1968
(Potonié 1931, Krutzsch, 1968)

1. *Labrapollis verrucatus* W. Kr. 1968 (Pl. II, fig. 23, 24) Présence: Sparnacien moyen: Chavot.

2. *Labrapollis labraferus* (R. Pot. 1931) W. Kr. 1968 (Pl. II, fig. 25—30) Présence: Sparnacien inférieur: Saint-Léger-aux-Bois 21/6—3; Sparnacien moyen: Sinceny 21/6—12.

3. *Labrapollis laevigatus* W. Kr. 1968 (Pl. II, fig. 31—34)
Présence: Sparnacien supérieur: Guitrancourt B_1—32; Sparnacien, Facies Argiles des Flandres: Templeuve-en-Pévele B_1—25; Cuisien supérieur: Cuise—2.

Fgen.: Zaklinskaiaepollenites n. fgen.

Fgen. type: *Zaklinskaiaepollenites concaviformis* (Zakl. 1963) n. comb.
Syn.: 1963. — Zaklinskaia, *Triatriopollenites concaviformis* n. fsp., pl. XL, 7.

Diagnose

Contour triangulaire, concave, coins arrondis. Exoapertures allongées dans la direction de l'axe polaire, les endoapertures sont des atria. Pas d'épassissement autour des exoapertures. Surface lisse ou scabre. Sous le niveau des atria les parties de l'exine extragerminale sont jointes par des plicae sur toute la surface des deux hemisphères.
Locus typicus: Bet-Pak-Dala (U. R. S. S.).
Stratum typicum: Aleurolit la sous-série de Djartaski.
Derivatio nominis: En hommage à Mme. Dr. E. D. Zaklinskaia qui a publié pour la première fois ce type de pollen.
Diagnose différentielle: Chez les genres *Plicapollis* Pf. 1953b, *Plicatopollis* W. Kr. 1962 emend. Frederiksen et Christopher 1978 les plicae ou d'autres différenciations de l'ectexine réjoignent toujours les régions aperturales. Le *Labrapollis* W. Kr. 1968 peut se distinguer en premier lieu par le contour equatorial, de plus, chez les pollens de ce nouveau genre de forme il n'y a jamais d'épaississement autour des exoapertures.

1. *Zaklinskaiaepollenites concaviformis* (Zakl. 1963) n. comb. (Pl. II, fig. 35—44)

Diagnose

Contour triangulaire concave, coins arrondis. Les exoapertures sont minces, de 0,5 à 1 µm. Surface scabre. L'exine extragerminale de 1 à 1,5 µm, l'épaisseur des trois couches de l'ectexine est égale. Les endoapertures sont larges (2—2,5 µm) et finement ponctuée. Les plicae extragerminales sont nettes.
Plus grande dimension: 15—22 µm.
Présence: Thanétien, zone II: Anizy-le-Château; Sparnacien inférieur: Saint Léger-aux-Bois 21/6—3; Sparnacien moyen: Boulogne-la-Grasse 21/6—18; Sparnacien supérieur: Guitrancourt B_1—32, Neuilly—46, Sinceny 21/6—9, 10, 11; Lutérien supérieur: Paris, Austerlitz.

Fgen.: *Plicatopollis* W. KR. 1962 emend. FREDERIKSEN et CHRISTOPHER 1978

Les résultats sur MeT et MeB de ce genre de forme soutiennent l'origine *Juglandanceae* de ces pollens (KEDVES et STANLEY 1976a). De plus il y a nombreux pollens avec des caractères intermédiaires entre *Plicatopollis* et *Platycaryapollenites*. C'est peut être ce qui explique que le *Plicatopollis pulcher* GRUAS-CAV. 1968 a été classé dans le genre *Platycaryapollenites* par FREDERIKSEN et CHRISTOPHER (1978). En se basant sur les caractères mentionnés plus haut (MeT et MeB), il faut supposer un genre fossil des *Juglandaceae,* qui est, vu leurs caractères, proche du genre *Platycarya*.

1. *Plicatopollis minor* KDS. 1974 (Pl. II, fig. 45, 46)
Présence: Sparnacien moyen: Boulogne-la-Grasse 21/6—18.
Appartenance botanique probable: *Juglandaceae.*

2. *Plicatopollis hungaricus* KDS. 1974 (Pl. II. fig. 47—50)
Présence: Lutétien supérieur: Paris, Austerlitz.
Appartenance botanique probable: *Juglandaceae.*

3. *Plicatopollis pulcher* GRUAS-CAV. 1968 (Pl. II, fig. 51—56).
Présence: Sparnacien inférieur: Saint Léger-aux-Bois 21/6—6a, Arpenty B_1—118; Sparnacien moyen: Boulogne-la-Grasse 21/6—18, Chavot; Sparnacien supérieur: Guitrancourt B_1—32; Sparnacien, Facies Argiles des Flandres: Templeuve-en-Pévele B_1—25, Watten B_1—6; Cuisien supérieur: Cuise—2; Lutétien supérieur: Paris, Austerlitz.
Appartenance botanique probable: *Juglandaceae.*

Fgen.: *Platycaryapollenites* E. NAGY 1969 emend. FREDERIKSEN et CHRISTOPHER 1978

Les données sur MeT et MeB concernant ce genre de forme ont été publiées par KEDVES et STANLEY (1976a). Espèces classées dans ce genre de forme:
Platycaryapollenites miocaenicus E. NAGY 1969.
Platycaryapollenites platycaryoides (ROCHE 1969) n. comb.
Syn.: 1969. — ROCHE, *Triatriopollenites platycaryoides* n. fsp., p. 135, pl. 1, 19.
1978. — FREDERIKSEN et CHRISTOPHER, *Platycarya platycaryoides* (ROCHE) n. comb., p. 138, pl. 3, 3—6.
Platycaryapollenites pseudoplatycaryoides (ROCHE 1969) n. comb.
Syn.: 1969. — ROCHE, *Triatriopollenites pseudoplatycaryoioides* n. fsp., p. 135, pl. 1, 18.
1978. — FREDERIKSEN et CHRISTOPHER, *Platycarya pseudoplatycaryoides* (ROCHE) n. comb., p. 138.
Platycaryapollenites swasticoidus (ELSIK 1974) FREDERIKSEN et CHRISTOPHER 1978
Platycaryapollenites triplicatus (ELSIK 1974) FREDERIKSEN et CHRISTOPHER 1978.

Platycaryapollenites dongyingensis KE et SHI 1978 in SUNG TZE CHEN et TSAO LIU, p. 105, pl. 34, 25–30. Syn.: 1953. – *Triatriopollenites coryphaeus* subsp. *microcoryphaeus* THOMSON et PFLUG.

Platycaryapollenites minutus KE et SHI 1978 in SUNG TZE CHEN et TSAO LIU, p. 106, pl. 34, 23, 24.

Platycaryapollenites shandongensis KE et SHI 1978 in SUNG TZE CHEN et TSAO LIU, p. 106, pl. 34, 31–33. Syn.: 1953. – *Triatriopollenites coryphaeus* subsp. *punctatus* THMSON et PFLUG.

Platycaryapollenites ferreri DE PORTA et al. 1985, p. 20, Lám. VII, figs. 11–20.

KRUTZSCH et VANHOORNE (1977) ont classé les pollens fossiles platycaryoides dans le genre de forme *Platycaryapollenites* W. KRUTZSCH 1969. Mais dans la bibliographie in n'y a aucune référence à ce sujet. L'étude, avec la description du genre de forme *Platycaryapollis* W. KR. n'a jamais été publiée, mais le manuscrit a été utilisé par quelques auteurs, par exemple: L. RÁKOSI (1973) – *Platycaryapollenites flagellus* W. KR. 1971; ZIEMBINSKA–TWORZYDLO (1974) – *Platycaryapollenites uformis* KRUTZSCH in press 1969, p. 373. Vu le travail de KRUTZSCH et VANHOORNE (1977) de ce point de vue, la situation est la suivante:

Platycaryapollenites saxonis W. KR. 1969, nomen nudum.

Platycaryapollenites trisolutionis (W. KR. et VANH. 1977) n. comb.

Syn.: 1977. – KRUTZSCH et VANHOORNE, *Platycaryapollis trisolutionis* n. fsp., p. 44, pl. 20, 4–7.

Platycaryapollenites levis (R. POT. 1931) n. comb. p. 3, Abb. 10. Basyonym: *Pollenites levis* R. POTONIÉ 1931; synonymes voir dans le travail de KRUTZSCH et VANHOORNE (1977).

Platycaryapollenites semicyclus (W. KR. et VANH. 1977) THIELE–PFEIFFER 1988.

Platycaryapollenites anticyclus (W. Kr. et VANH. 1977) n. comb.

Syn.: 1977. – KRUTZSCH et VANHOORNE, *Platycaryapollis anticyclus* n. fsp., p. 45, pl. 20, 19–21, cf. 17–18.

Platycaryapollenites irregularis (W. KR. et VANH. 1977) n. comb.

Syn.: 1977. – KRUTZSCH et VANHOORNE, *Platycaryapollis irregularis* n. fsp., p. 45, pl. 20, 22–26.

1. *Platycaryapollenites miocaenicus* E. NAGY 1969 (Pl. II, fig. 57–64)

Présence: Thanétien, zone II: Anizy-le-Château, Rollot 21/6–16, Thanétien, zone III: Rollot 21/6–17; Sparnacien inférieur: Saint Léger-aux-Bois 21/6–6a, 21/6–3, Arpenty B_1–118; Sparnacien moyen: Boulogne-la-Grasse 21/6–18, Chavot, Sinceny 21/6–12; Sparnacien supérieur: Neuilly–46, Neuilly–37, Sinceny 21/6–7, 8, 21/6–9, 10, 11; Sparnacien, Facies Argiles des Flandres: Watten B_1–6; Cuisien supérieur: Cuise–2, Fosses I–III; Lutétien supérieur: Paris, Austerlitz.

Appartenance botanique probable: *Juglandaceae, Platycarya.*

2. *Platycaryapollenites levis* (R. POT. 1931) n. comb. (Pl. II, fig. 65–70).

Présence: Thanétien, zone II: Rollot 21/6–16; Sparnacien inférieur: Saint Léger-aux-Bois 21/6–3; Sparnacien moyen: Boulogne-la-Grasse 21/6–18, Chavot,

Sinceny 21/6—12; Sparnacien supérieur: Neuilly—46, Neuilly— 37, Sinceny 21/6—7, 8, 21/6—9, 10, 11; Sparnacien, Facies Argiles des Flandres: Watten B_1—6; Cuisien supérieur: Cuise—2, Fosses I—III.
Appartenance botanique probable: *Juglandaceae, Platycarya.*

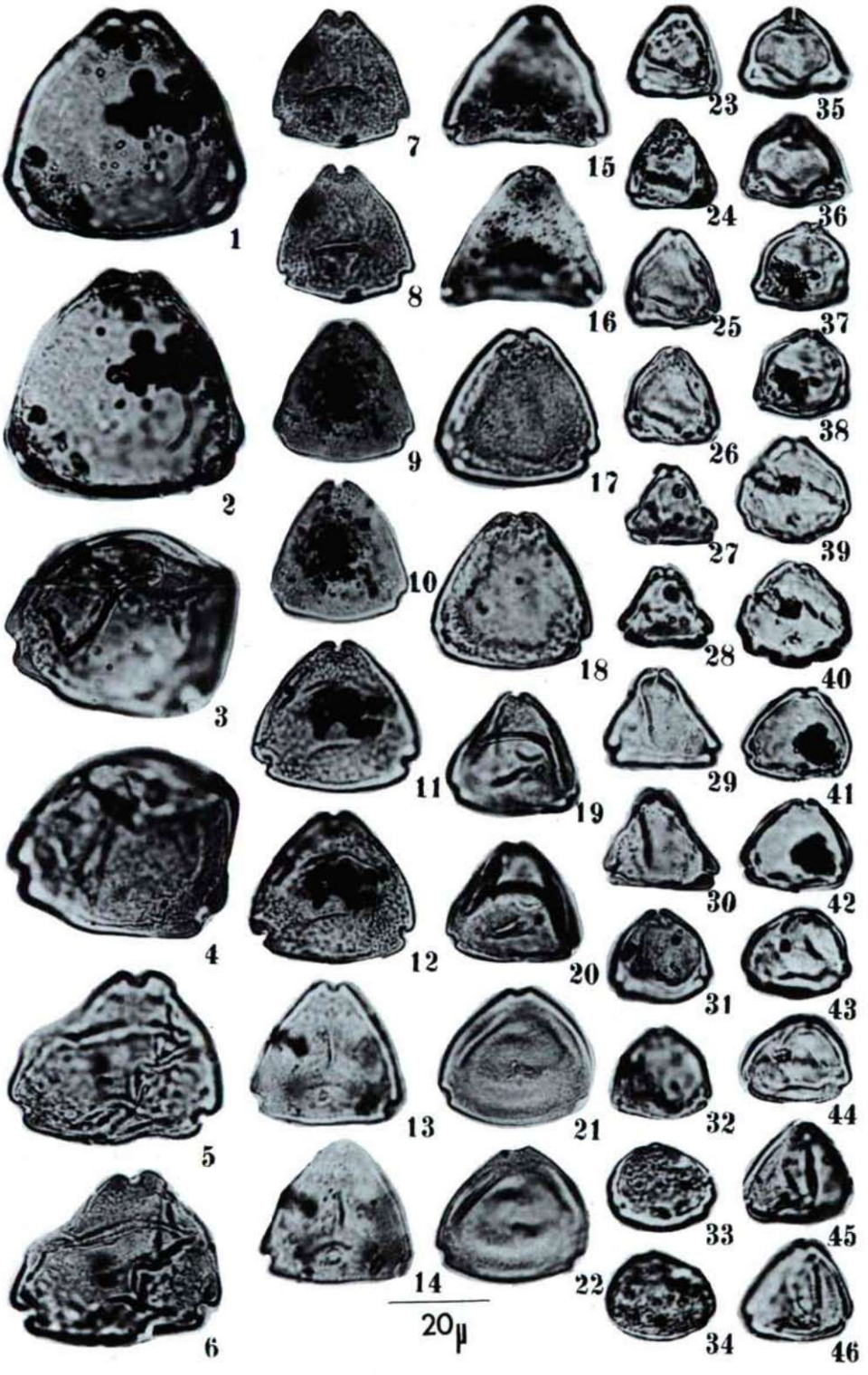
3. Platycaryapollenites platycaryoides (ROCHE 1969) n. comb. (Pl. II, fig. 71—80).
Présence: Thanétien, zone II: Anizy-le-Château, Rollot 21/6—16; Sparnacien inférieur: Saint Léger-aux-Bois 21/6—6a, Arpenty B_1—118; Sparnacien moyen: Boulogne-la-Grasse 21/6—18, Chavot, Sinceny 21/6—12; Sparnacien supérieur: Guitrancourt B_1—32, Neuilly—46, Neuilly—37, Sinceny 21/6—7, 8, 21/6—9, 10, 11; Sparnacien, Facies Argiles des Flandres: Templeuve-en-Pévele B_1—25; Cuisien supérieur: Troesnes I—III, Cuise—2; Lutétien supérieur: Paris, Austerlitz.
Appartenance botanique probable: *Juglandaceae, Platycarya.*

4. *Platycaryapollenites anticyclus* (W. KR. et VANH. 1977) n. comb. (Pl. II, fig. 81—88).
Présence: Thanétien, zone II: Anizy-le-Chateau; Sparnacien inférieur: Saint-Léger-aux-Bois 21/6—6a; Sparnacien moyen: Chavot, Sinceny 21/6—12; Sparnacien supérieur: Guitrancourt B_1—32, Neuilly—46, Neuilly—37, Sinceny 21/6—7, 8, 21/6—9, 10, 11; Sparnacien, Facies Argiles des Flandres: Templeuve-en-Pévele B_1—25; Cuisien supérieur: Troesnes I—III.
Appartenance botanique probable: *Juglandaceae, Platycarya.*

à suivre

Plance I

1,2. *Triatriopollenites roboratus* PF. 1953a, *Myricaceae,* prep. AT—14; 89,8/7,6.
3,4. *Triatriopollenites roboratus* PF. 1953a, *Myricaceae,* prep. Chavot 1/1; 88,3/4,4.
5,6. *Triatriopollenites roboratus* PF. 1953a, *Myricaceae,* prep. 21/6—18, 86,7/5,2.
7,8. *Alabroidaepollenites aroboratus* (PF. 1953a) KDS. et RUSS. 1982, *Myricaceae,* prep. 21/6—6a—5; 91,2/19,4.
9,10. *Alabroidaepollenites aroboratus* (PF. 1953a) KDS. et RUSS. 1982, *Myricaceae,* prep. 21/6—16—1/1, 91,2/3,0.
11,12. *Alabroidaepollenites aroboratus* (PF. 1953a) KDS. et RUSS. 1982. *Myricaceae,* prep. B_1—32—1; 89,2/20,2.
13,14. *Alabroidaepollenites aroboratus* (PF. 1953a) KDS. et RUSS. 1982, *Myricaceae,* prep. 21/6—16—1/8, 98,8/7,1.
15,16. *Alabroidaepollenites aroboratus* (PF. 1953a) KDS. et RUSS. 1982, *Myricaceae,* prep. 21/6—16—1/4; 87,3/23,7.
17,18. *Alabroidaepollenites aroboratus* (PF. 1953a) KDS. et RUSS. 1982, *Myricaceae,* prep. N—37—L—183—2c—118—1; 99,6/4,2.
19,20. *Labraferoidaepollenites pseudorurensis* (PF. 1953a) KDS. et RUSS. 1982, *Myricaceae,* prep. Nointel 2a; 105,2/4,3.
21,22. *Labraferoidaepollenites pseudorurensis* (PF. 1953a) KDS. et RUSS. 1982, *Myricaceae,* prep. Nointel 2c; 95,8/6,5.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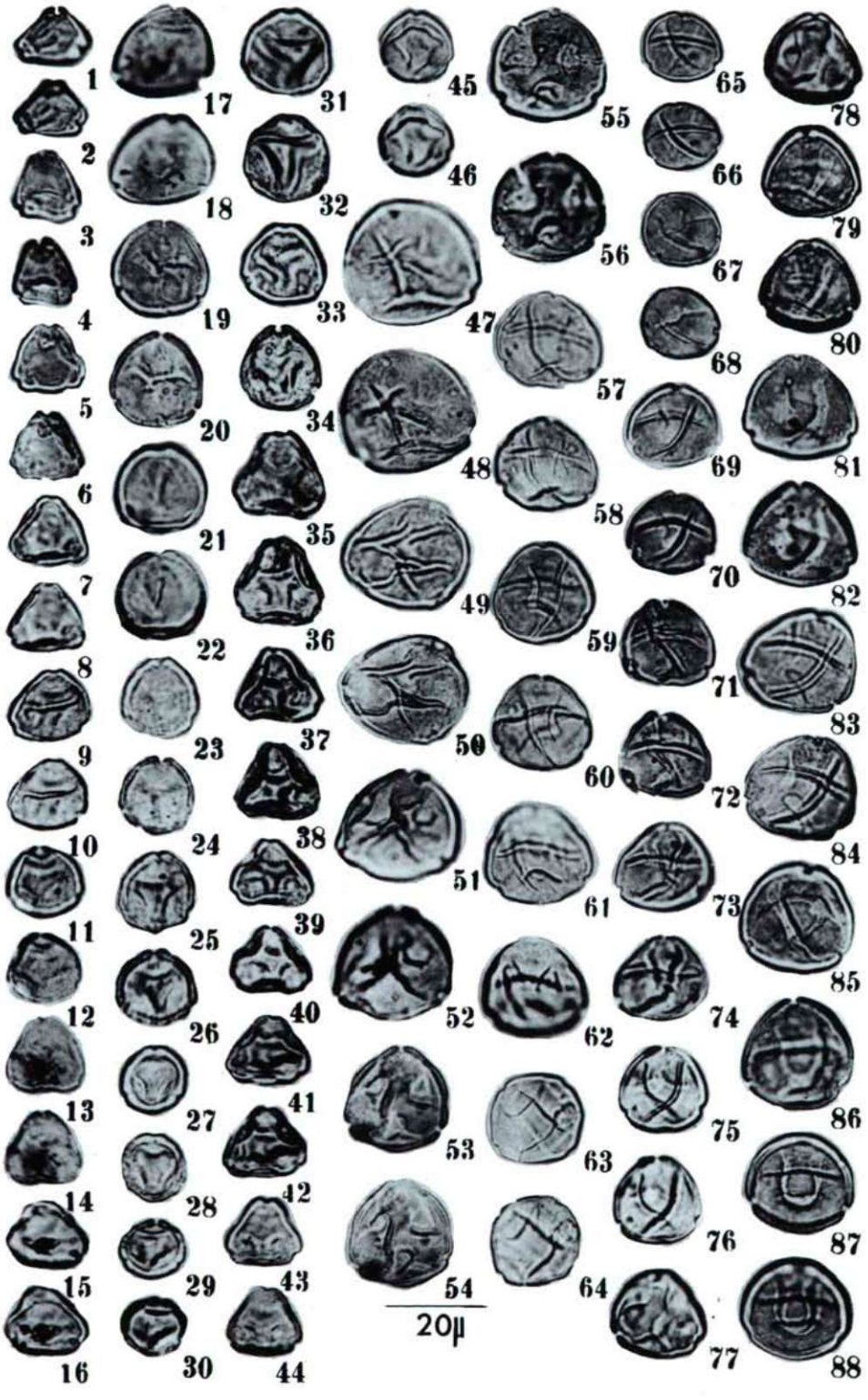
23,24. *Labraferoidaepollenites conspicuus* (GLADK. 1965) KDS. 1974, *Myricaceae*, prep. B_1-32-1; 96,2/13,1.
25,26. *Labraferoidaepollenites conspicuus* (GLADK. 1965) KDS. 1974, *Myricaceae;* prep. 21/6–18; 85,1/17,4.
27,28. *Labraferoidaepollenites conspicuus* (GLADK. 1965) KDS. 1974, *Myricaceae*, prep. AT–18; 95,9/16,2.
29,30. *Labraferoidaepollenites conspicuus* (GLADK. 1965) KDS. 1974, *Myricaceae*, prep. Chavot 1/1; 98,7/9,4.
31,32. *Alabroidaepollenites paleogenicus* (BOLOTNIKOVA 1975) n. comb., *Juglandaceae, Engelhardtia*, prep. B_1-6-6; 105,6/3,9.
33,34. *Alabroidaepollenites paleogenicus* (BOLOTNIKOVA 1975) n. comb., *Juglandaceae, Engelhardtia*, prep. B_1-32-1; 110,1/14,2.
35,36. *Triatriopollenites sauerae* (GLADK. 1965) KDS. 1974, *Myricaceae*, prep. 21/6–16–1/2; 84,3/19,1.
37,38. *Triatriopollenites sauerae* (GLADK. 1965) KDS. 1974, *Myricaceae*, prep. Troesnes–III/2; 91,2/6,8.
39,40. *Labraferoidaepollenites intermedius* (GLADK. 1965) n. comb., *Myricaceae*, prep. AT–2; 82,8/22,0
41,42. *Labraferoidaepollenites intermedius* (GLADK. 1965) n. comb., *Myricaceae*, prep. AT–2; 99,3/4,3.
43,44. *Labraferoidaepollenites intermedius* (GLADK. 1965) n. comb., *Myricaceae*, prep. AT–2; 99,4/5,1.
45,46. *Labraferoidaepollenites intermedius* (GLADK. 1965) n. comb., *Myricaceae*, prep. AT–15; 96,2/24,1.

Planche II

1,2. *Labraferoidaepollenites dilatus* (FAIRCHILD. 1966) KDS. et RUSS. 1982, *Juglandaceae*, cf. *Engelhardtia*, prep. N–46–L–183–2c–118–2; 88,2/14,1.
3,4. *Labraferoidaepollenites dilatus* (FAIRCHILD. 1966) KDS. et RUSS. 1982, *Juglandaceae*, cf. *Engelhardtia*, prep. Austerlitz 1/1; 88,8/6,9.
5,6. *Labraferoidaepollenites dilatus* (FAIRCHILD. 1966) KDS. et RUSS. 1982, *Juglandaceae*, cf. *Engelhardtia*, N–46–L–183–2c–118–2; 87,8/14,2.
7,8. *Labraferoidaepollenites dilatus* (FAIRCHILD. 1966) KDS. et RUSS. 1982, *Juglandaceae*, cf. *Engelhardtia*, prep. 21/6–18; 89,4/2,9.
9,10. *Triatriopollenites minimus* (GLADK. 1965) KDS. 1974, *Juglandaceae, Engelhardtia*, prep. 21/6–18; 94,7/3,4.
11,12. *Triatriopollenites minimus* (GLADK. 1965) KDS. 1974, *Juglandaceae, Engelhardtia*, prep. 21/6–18; 77,1/2,8.
13,14. *Triatriopollenites minimus* (GLADK. 1965) KDS. 1974, *Juglandaceae, Engelhardtia*, prep. 21/6–16–1/4; 104,1/12,0.
15,16. *Triatriopollenites minimus* (GLADK. 1965) KDS. 1974, *Juglandaceae, Engelhardtia*, prep. AT–13; 107,4/8,5.
17,18. *Triatriopollenites engelhardtioides* (ROCHE 1973) ROCHE et SCHULER 1976, *Juglandaceae, Engelhardtia*, prep. 21/6–16–1/6; 96,1/24,1.
19,20. *Triatriopollenites engelhardtioides* (ROCHE 1973) ROCHE et SCHULER 1976, *Juglandaceae, Engelhardtia*, prep. 21/6–12; 87,4/2,2.
21,22. *Momipites kedvesii* (GRUAS–CAV. 1967) FFEDERIKSEN et CHRISTOPHER 1978, prep. 21/6–7; 99,2/14,5.
23,24. *Labrapollis verrucatus* W. KR. 1968, prep. Chavot 1/2; 77,2/18,7.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25,26. *Labrapollis labraferus* (R. POT. 1931) W. KR. 1968, prep. 21/6—12; 93,2/2,1
27,28. *Labrapollis labraferus* (R. POT. 1931) W. KR. 1968, prep. 21/6—12; 96,8/6,6
29,30. *Labrapollis labraferus* (R. POT. 1931) W. KR. 1968, prep. 21/6—12; 92,7/4,6
31,32. *Labrapollis laevigatus* W. KR. 1968, prep. Cuise—2/2; 89,8/9,4.
33,34. *Labrapollis laevigatus* W. KR. 1968, prep. B_1—32—1; 99,8/14,2.
35,36. *Zaklinskaiaepollenites concaviformis* (ZAKL. 1963) n. fgen. n. comb., prep. 21/6—3d; 84,3/14,3.
37,38. *Zaklinskaiaepollenites concaviformis* (ZAKL. 1963) n. fgen. n. comb., prep. 21/6—3b; 78,6/2,6.
39,40. *Zaklinskaiaepollenites concaviformis* (ZAKL. 1963) n. fgen. n. comb., prep. 21/6—18; 80,7/22,5.
41,42. *Zaklinskaiaepollenites concaviformis* (ZAKL. 1963) n. fgen. n. comb., prep. N—46—L—183—2c—118—2; 90,7/5,6.
43,44. *Zaklinskaiaepollenites concaviformis* (ZAKL. 1963) n. fgen. n. comb., prep. 21/6—10; 97,2/8,5.
45,46. *Plicatopollis minor* KDS. 1974, *Juglandaceae*, prep. 21/6—18; 91,7/11,0.
47,48. *Plicatopollis hungaricus* KDS. 1974, *Juglandaceae*, prep. Austerlitz 1/1; 88,7/17,1.
49,50. *Plicatopollis hungaricus* KDS. 1974, *Juglandaceae*, prep. Austerlitz 1/1; 83,4/9,4.
51,52. *Plicatopollis pulcher* GRUAS-CAV. 1968, *Juglandaceae*, prep. Austerlitz 1/3; 96,7/17,4.
53,54. *Plicatopollis pulcher* GRUAS-CAV. 1968, *Juglandaceae*, prep. Chavot 1/2; 75,0/20,2.
55,56. *Plicatopollis pulcher* GRUAS-CAV. 1968, *Juglandaceae*, prep. B_1—32—2; 84,4/2,9.
57,58. *Platycaryapollenites miocaenicus* E. NAGY 1969, *Juglandaceae, Platycarya*, prep. 21/6—18; 106,2/2,3.
59,60. *Platycaryapollenites miocaenicus* E. NAGY 1969, *Juglandaceae, Platycarya*, prep. 21/6—18; 78,3/3,0.
61,62. *Platycaryapollenites miocaenicus* E. NAGY 1969, *Juglandaceae, Platycarya*, prep. 21/6—18; 80,1/13,7.
63,64. *Platycaryapollenites miocaenicus* E. NAGY 1969, f. tetraexitum. n.f., *Juglandaceae, Platycarya*, prep. 21/6—18; 100,0/14,2.
65,66. *Platycaryapollenites levis* (R. POT. 1931) n. comb., *Juglandaceae, Platycarya*, prep. 21/6—18; 89,7/2,8.
67,68. *Platycaryapollenites levis* (R. POT. 1931) n. comb., *Juglandaceae, Platycarya*, prep. 21/6—18; 87,4/2,8.
69,70. *Platycaryapollenites levis* (R. POT. 1931) n. comb., *Juglandaceae, Platycarya*, prep. Cuise 2/1; 98,9/20,2.
71,72. *Platycaryapollenites platycaryoides* (ROCHE 1969) n. comb., *Juglandaceae, Platycarya*, prep. 21/6—62—2; 106,3/6,6.
73,74. *Platycaryapollenites platycaryoides* (ROCHE 1969) n. comb., *Juglandaceae, Platycarya*, prep. B_1—118—4; 89,6/3,2.
75,76. *Platycaryapollenites platycaryoides* (ROCHE 1969) n. comb., *Juglandaceae, Platycarya*, prep. B_1—32—1; 116,2/19,3.
77,78. *Platycaryapollenites platycaryoides* (ROCHE 1969) n. comb., *Juglandaceae, Platycarya*, prep. N—46—L—183—2c—118—2; 95,0/2,5.
79,80. *Platycaryapollenites platycaryoides* (ROCHE 1969) n. comb., *Juglandaceae, Platycarya*, prep. N—37—L—183—2c—118—1; 91,9/6,4.
81,82. *Platycaryapollenites anticyclus* (W. KR. et VANH. 1977) n. comb., *Juglandaceae, Platycarya*, prep. 21/6—6a—1; 102,4/3,2.
83,84. *Platycaryapollenites anticyclus* (W. KR. et VANH. 1977) n. comb., *Juglandaceae, Platycarya*, prep. Chavot 1/1; 79,1/7,9.
85,86. *Platycaryapollenites anticyclus* (W. KR. et VANH. 1977) n. comb., *Juglandaceae, Platycarya*, N—46—L—183—2c—118—2; 86,1/4,3.
87,88. *Platycaryapollenites anticyclus* (W. KR. et VANH. 1977) n. comb., *Juglandaceae, Platycarya*, prep. B_1—32—1; 84,6/14,3.

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POLLINATION BIOLOGY OF SOUR CHERRY VARIETIES OF PROTOGYN BLOSSOMING

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Abstract

Protogyny as one type of dichogam mechanisms supposes mostly autosterility. Yet in sour cherry varieties protogyny occurs both in autosterile and autofertile types. In some of the autosterile types the receptivity of stigma and the phase of opening of anthers is totally separated. A true dichogamy is indicated by a 12 hour periodicity of nectar secretion and the degeneration of stigma papills in the young opened flower. In the phase of opening of anthers the 12 hour diurnal rhithm of nectar production remains, but it is shifted by 6 hours. In the autofertile types protogyny is indicated by the stigma exerted or by the stigma of pollination chamber which is accompanied by a 12 hour secretion rhithm characteristic for dichogamy. Protogynous flowers waiting for extraneous pollination at the beginning of blossoming avoid selfpollination by dichogamy, but during the opening of anthers they become homogamious indicated by a nectar secretion rhithm with 6 hour periodicity and by the delayed receptivity of stigma. Such "delayed" homogam flowers can be fertilized by self pollination at the end of blossoming. The changing of pollination strategy is characteristic for the protogyn sour varieties: 1. stigma exerted – wind pollination, 2. state of pollination chamber – beetle pollination, 3. opening of anthers – pollination by bees and other insects.

Key words: sour cherry varieties, sour cherry pollination biology, protogyny, periodicity of nectar secretion.

Introduction

Most of the authors describe protogyny as a dishogam mechanism which excludes self-pollination (STOUT, 1928, SCHROEDER, 1943; SPENCER–KENNARD, 1955; THIEN, 1974; SEDLEY, 1977). In their opinion the protogynous flower is pollinated or in green bud stage, in stigma exerted stage streaching out of the green bud, or one day after the opening of the flower. PILJ (1961) and GOTTSBERGER (1974, 1977) emphasize the importance of cantharophyly, the pollination chamber stage and the role of beetle pollination in the pollination of protogyn *Prunus* and *Pyrus* types showing a secondary polyandry. Studying the turgorous stae of cherry, plum and apple stigma papillies STÖSSER (1985) considered the day of blossoming as optimal from the point of view of pollination.

The flowers of sour cherry varieties were described by Mohácsi and Maliga (1956) as protogyn. Their observations were supported by Nyéki (1974), but he experienced homogamy too. Proterandry is rare in sour cherry varieties, but this phenomenon was found in one case (Orosz Kovács et al., 1987). In Pejkic's opinion receptivity of stigma in Pándy sour cherries lasts for 1—2 days after blossoming. Nyéki and Ifjú (1975) discovered that the stigma secretion activity and the daily rhithm of the opening of anthers are different in the varieties studied by them.

A summary about the control of floral nectar secretion is given by Bently and Elias (1983). The periodicity of nectar production of *Prunoideae* taxons and the synchronization of the endogen rhithm with stigma receptivity and anther dehiscence were studied by Orosz Kovács (1988, 1990), Orosz Kovács et al. (1987, 1988, 1989), Majer Bodrács et al. (1989). They found that dichogam flowers produce nectar periodically by 12 th hours, the homogam ones by 6 th hours, and the time of maximum production is synchronized by the stigma receptivity and anther dehiscence. When studying the periodicity of nectar production of the Pándi sour cherry clones we differentiated three types of floral secretion on the basis of the highest production values (Orosz Kovács et al., 1989).

Material and Method

We carried out the examination of the floral nectar secretion of protogyn sour cherry varieties between 1988—1991 at the Research Station of Fruit — and Ornament Plant Growing and Research Company in Cegléd and in the orchard in Ceglédbercel belonging to the South-Pest County Stone-fruit Growing Company. We observed with attention the development of stigma surface of the Érd type with large fruit from the green bud stage to the opened flower. The beginning of nectar secretion, the formation of nectary surface in the same stages of development were studied in the M—18 protogyn sour cherry type. To examine the stigma and nectary surface the fresh material was fixed in 3% glutaraldheid, and the washing was done in 0,1 mol. Na-kakodilat puffer. The fixed material was dehydrated in ethylic alcohol series. At a critical point drying and gold shadowing were done. The micro-photos were taken with help of ASID—4 SEM adapted to yeol 100 — C in 1000—10 000 magnification.

Results

The changing of pollination strategy is characteristic for sour cherries with protogyn flowers. Two forms of function ability of stamina and pistil can be found in them, namely true dichogam protogyny and "delayed" homogamy (pseudodichogamy) beginning with protogyny.

The flowers of sour cherry of Érd type with large fruit are true protogyn. The stigma is already mature 6—10 days before blossoming. On the exerted stigma surface streching out of the green bud stigma secretion can be observed at 3 and 15 o'clock — according to the time of secretion rhithm. The receptivity of stigma begins already at a 3—5 mm size of the bud. The stigma papills are

already totally developed in their green bud stage (Fig. 7—8.), their surface is turgorous, the stigma surface is mature for pollination. The reproduction organ in this stage of development is not attractive for the insects at all. The stamina in the closed bud are unmature, nectar secretion does not begin yet. On the nectary surface we can see that the stomas through which the fluid of secretion emerges are closed, the cuticle has not burst open yet (Fig. 1.). The only way of pollination is: the wind as mediatory. The stigma exerted and the relatively large stigma surface also refer to this fact. The glitter of stigma surface may indicate some beetle attraction but no beetles were seen on the stigma streching out of the bud during the time of examination.

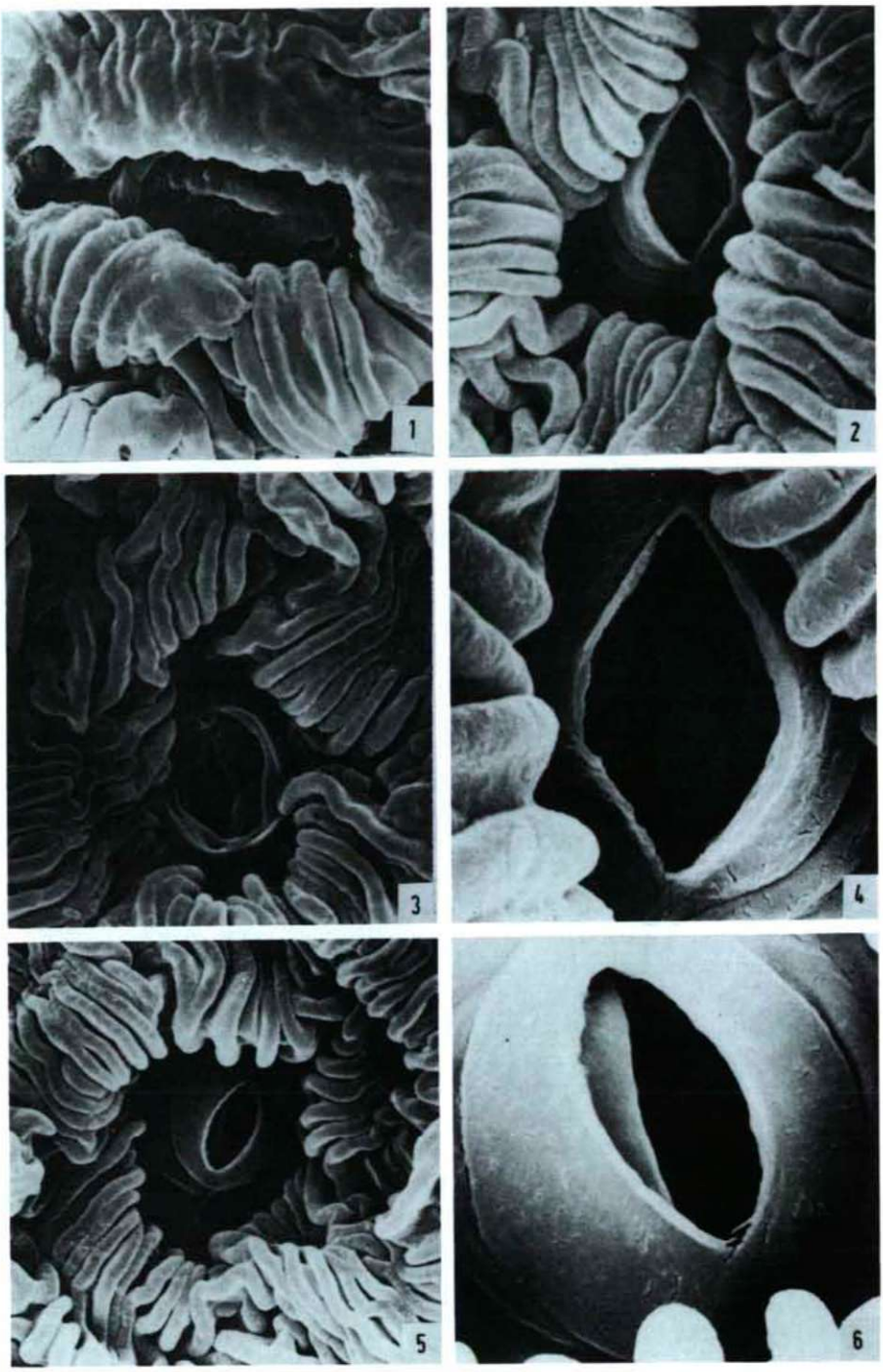
The degeneration of stigma papills begins unusually very soon. At the end of the green bud stage papills lose from their turgors, slight creases (crumplings) can be seen (Fig. 9.) but they cannot possibly prevent pollination.

The green bud stage is soon floowed by the "pollination chamber" stage. The petals grow on and reach the hight of stigma where they form a circle like orifice giving room for the stigma to drive in. During the time of "pollination chamber" stage — which in GOTTSBERGER's opinion (1977) is characteristic for each of the protogyn *Prunus* and *Pyrus* varieties — the pollination is done by cantharofil beetles. In our observations the scent of flowers of the pollination chamber in the sour cherry of Érd variety with large fruit resembels that of rotting fruit and the same findings were described by PILJ (1961) and GOTTSBERGER (1974, 1977). The scent exciting the cantharofil beetles, as well as the characteristic white colour of the flowers (petals) and the green one (nectary, stigma) confirm the possibility of beetle pollination. In the opinion of the authors mentioned before beetles living in the flower of openig bud stage feed themselves with stigma secretion, nectar, pollen, petals and may significantly damage the flowers. The pollination is possibly carried out by pollen stuck to the beetles legs wet with stigmatic secretion (GOTTSBERGER, 1977).

Nectar secretion in the flower begins in the "pollination chamber" stage. Secretion rushing up through the stoma lifts and tears the cuticle covering the nectary stoma (Fig. 2—4.). The "pollination chamber" stage may go on for 1—3 days depending on the weather. In this flower stage the nectar is very diluted, the dry matter is below 10%. Secretion of so low calori value is not attractive at all for the bees, and they did not attend flowers of opening bud stage. A considerable number of smaller beetles can be seen in buds of this stage.

By the end of the "pollination chamber" stage the stigma papills shrivel (Fig. 10.) and when the flower opens the stigma surface is not suitable for the reception of pollen.

Nectar secretion which started in the "pollination chamber" stage of stigma phase works by a regular rhithm. Nectar is produced twice a day at 03 and 15 o'clock, that is in a 12 hour interval (Fig. 11.). The secretion at dawn attracts night insects while that early in the afternoon the daily ones. In the young, opened flowers the stigma is already turning brown, but the nectar is still delute, not attractive for the bees. If the weather is cool, it may happen that the insects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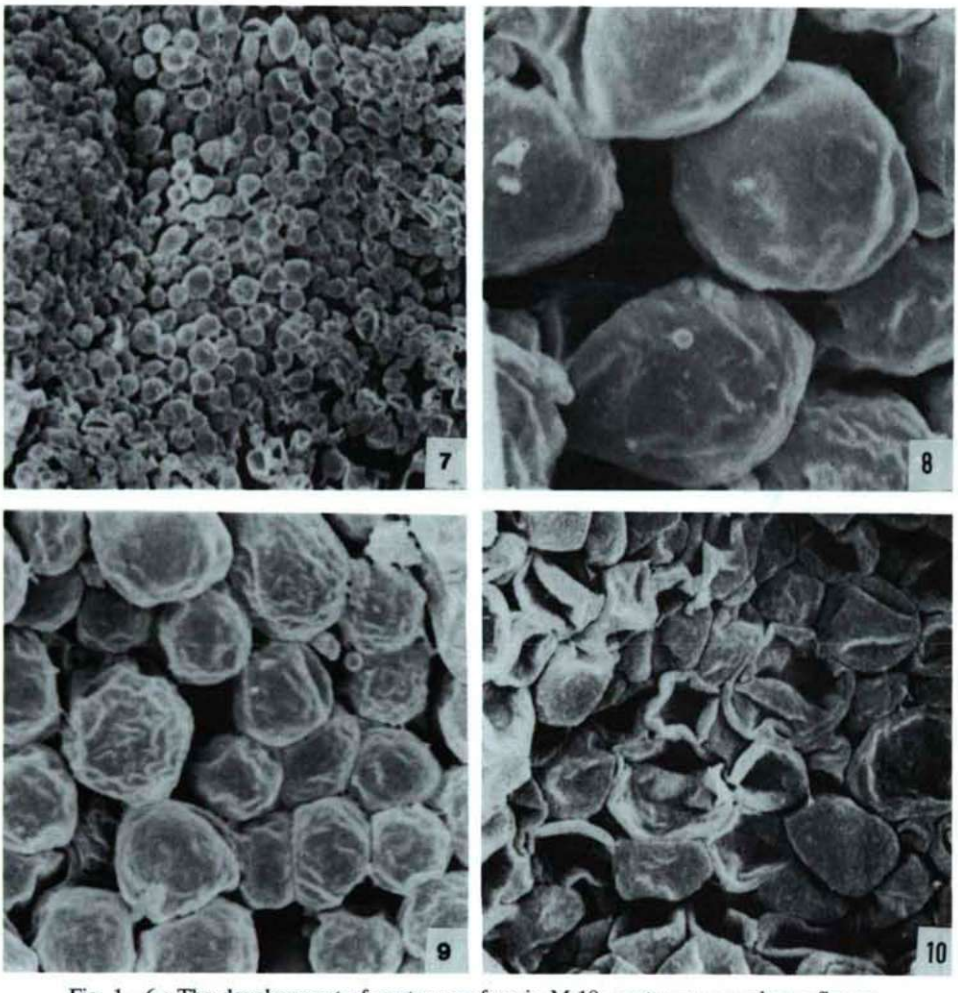

Fig. 1—6.: The development of nectary surface in M 18. protogyn sour cherry flower during blossoming.

1. In closed, green bud stage: the closing cells of the stoma have not opened yet, the cuticle covering the stoma has not torn (SEM 300 x)

2—4. In the pollination chamber stage when nectar secretion begins, the cuticle tears above the stoma, the closing celles become visible (SEM 3000 x, 10 000 x).

5—6. The stoma of nectaries in the flower with open anthers is open (SEM 3000 x, 10 000 x).

Fig.. 7—10. : The changing of stigma surface in the protogyn flower of Érd sour cherry with large fruit during the development of the flower.

7—8. In green bud stage the papills of stigma exerted are wholly developed, trugorous, (SEM 300 x, 3000 x).

9. By the and of the green bud stage the surface of papills begins to crease (SEM 1000 x).

10. During the formation of pollination chamber a significant part of the stigma surface died (SEM 1000 x).

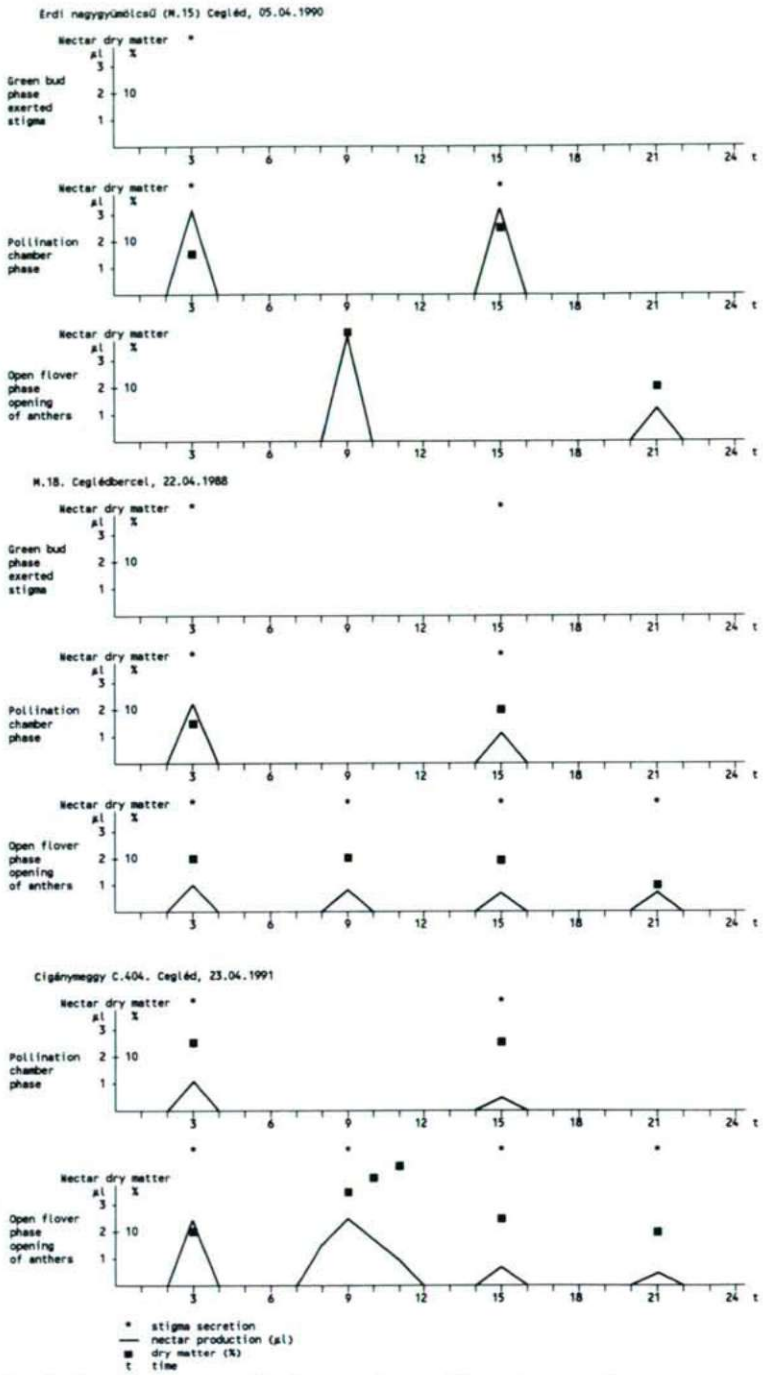

Fig. 11. The rhythm of nectar secretion in sour-cherry with protogynous flowers.

do not consume the secretion produced in the bud and during 2—3 days it is concentrated so much that the dry matter exceeds 10%. In warm weather (about 20 C°) the dry matter of the young flower secretion exceeded 20% in some cases. This time it may happen that the bees visiting the flowers for nectar touch the stigma when streching down to the receptacle through the nectar guide and it may be probable that successful fertilisation can still be realized in this young flower stage described by STÖSSER (1985).

The flowers of sour cherry of Érd variety with large fruit are visited by bees mostly during the opening of anthers. This time the stigma surface is wholly brown. The flower excludes self pollination with true dichogamy. Now the bees have a role not in supplying the stigma with pollens but in sending them away. During the opening of anthers the floral secretion rhithm changes. Nectar is produced by 12 hours as previously (Fig. 11.) but the production maximums are shifted by 6 hours, i. e. secretion takes palce at 09 and 21 o'clock, one at daytime one at night. The nectar concentration is already suitable for the bees and the flowers are visited beside bees by other insects too.

The flowers of Érd autosterile sour cherry variety with large fruit change pollination strategy three times. At the beginning of blossoming they avoid selfpollination with the earlier mature stigma exerted and due to the lack of insect attraction they are pollinated by wind. In the "pollination chamber" stage beetle pollination comes into prominence and finally the third changing of strategy takes place at the opening of the flower, when during the opening of anthers the pollen transport by bees and different insects takes place. The pollination of flowers of this variety may be done essentially during the first two stages.

The protogyn of M. 18 sour cherry flowers is partially different from the previously described one. The first strategy here too is based on pollination by wind in green bud stage, in exerted stigma state. The "pollination chamber" stage ensuring the conditions of beetle pollination can be found too. The difference, if compared with the previous variety, is that the stigma retains its receptivity during the whole life of the flower. So during the opening of anthers the stigma has still its vitality, and as a autofertile type, it may be fertilized by its own pollens. The aim of the changing of strategy here is to bring about genetic refreshment, and in case of failure it shows the direction of getting pollens by all means.

The rhithm of nectar secretion indicates the first separated then the joint operation of stamina and the pistil. Similary to the dichogam flowers of the Érd variety with large fruit here nectar production begins also in the pollination chamber stage at 03 and 15 o'clock (Fig. 11.). In the opened flowers, however, in the time of opening of anthers the 6 hour rhithm characteristic for homogamous flowers (works), i. e. the production maximums appear at 03, 09, 15 and 21 o'clock. According to the autofertile varieties the amount of secretion is low.

The flowers of M.18 sour cherry clone are not really true dichogam. The initial separation of stamina and pistil is soon followed by homogamy. The

synchronized, joint work of genital leaves entering later is called "delayed" homogamy.

Similary "delayed" homogamy can be seen in the C. 404 clone of Cigánymeggy (Gipsy sour cherry) whose flowers do not have the characteristic exerted stigma stage. The maturity of flower suitable for pollination begins in the "pollination chamber" stage and after the dichogam phase the stamen and pistil turn into homogamy during the opening of anthers (Fig. 11.). The autofertile type is pollinated with extraneous pollens during blossoming. If it does not occur, self-fertilisation may take place in the homogam phase. The latter phenomenon can be observed at the end of blossoming when the mature anthers very often hang over the stigma. We observed these "delayed" homogam flowers in our earlier work too, e. g. similar phenomenon was experienced in the variety of Újfehértó racemose (Orosz Kovács et al. 1989.).

Each of the above mentioned varieties had the same secretion rhithm, the maximums of nectar production — synchronized with the opening of anthers and receptivity of stigma were at 3, 9, 15 and 21 o'clock. Summerizing our results it can be said that the pollination of protogyn sour cherry varieties may be realized by multi-changing of pollination strategy: e. g. in green bud stage, in stigma exerted stage by wind, and finally in pollination chamber stage by beetles and finally in the open flower with open anthers by bees and other insects. Protogyny occurs in both autosterile and autofertile varieties. In some autosterile varieties the phase of receptivity of stigma and the opening of anthers is totally separated. During the time of blossoming the stigma surface is already degenerated and besides the 12 hour periodicity of nectar secretion also indicates dichogamy. In the autofertile varieties protogyny is indicated by the stigma exerted or the pollination chamber stage accompanied by a 12 hour dichogam secretion rhithm. Dichogamy is characteristic only during blossoming, the flowers turn into homogamy at the opening of anthers which can be seen in the 6 hour nectar secretion rhithm and the prolonged receptivity of stigma. These flowers of "delayed" homogamy (pseudohomogamy) may be fertilized by self-pollination at the end of blossoming.

The quick changing of pollination strategy of flowers, variability in one fruit type make the application of flower biological observations justified in the planning of up-to date mixed planting of the fruit trees.

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BEHAVIOUR OF AN ALCOHOL-PREFERRING STRAIN OF WISTAR RATS

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Abstract

The 16th generation of alcohol preferring (P) and non-preferring (NP) rats was used for behavioural experiments and synaptological studies. In the open-field test, the male P animals showed more inner ambulations, groomings and wall rearing with shorter latencies and a lower defecation rate with longer latency. The P females did more ambulations and their motility was higher in the inner part of the open field. They showed more wall rearing and grooming than the NP females with shorter latencies. The defecation rate in the P group was lower, although its latency was longer on the first day. In the time-to-emerge test the latency of emergence of the P males was longer than that of the NPs, but not that of the P females. In the plus-maze test the latency of leaving the centre was short for all the P animals and the P males more often entered into the open arm. Both the female and male P animals spent less time there than the NPs. The defecation rate of the P rats was higher. The EM studies revealed a significant decrease in the number of synapses on pyramidal cell apical dendrites in layer 4 of the cerebral cortex and this parameter also differed – although not significantly – in the molecular layer of the hippocampus in the P rats, while an increase in the synapse density was seen in the molecular layer of the cerebellar cortex in the P strain. The results showed that the behavioural pattern of the P animals is not fear-motivated but rather non-adaptive in the stress situations. Since the parameteres observed in this study can be correlated with the behavioural elements of the human alcoholics, this animal model seems to be useful in studies of alcoholism.

Key words: rats, alcohol preference, open-field test, time to emerge test, plus maze test, synapse counting.

Introduction

Several laboratories have described the effects of acute and chronic ethanol administration both in animal experiments and in clinical investigations (e. g. MILLER, 1986; WARTBURG, 1979; WOOD et al., 1987). Most of these investigations are concerned with the ethanol metabolism in the liver (e. g. ROVINSKY et al., 1987) and dysfunction of the central nervous system(CNS) (e. g. CHAMACHO–NASI and TREISTMANN, 1986; MILLER, 1986; SIGGINS et al., 1986; WOOD and SCHROEDER, 1988). The other line of the experimental research has tried to elucidate the long term events, that might affect the structural organization (BAUER–MAFFETT and ALTMAN, 1977; BERACOCHEA et al., 1987; HOFF, 1988),

ongoing physiological processes in the CNS (GORDON et al., 1986; MORZORATI et al., 1988) and even the inherited genetic information (GOLDMAN et al., 1985; GOLDMAN et al., 1987; GOODWIN et al., 1974; OLIVERIO and ELEFTHERIOU, 1976). LI et al. (1986) were the first to report, that generations of an alcohol-preferring (P) line of rats were bred out. The behavioural properties of this inbred strain were examined and found to be different when compared with the nonpreferring (NP) rats.

On the basis of these experiments, an inbred P strain of Wistar rats was also derived in our laboratory (SCHULZ, 1987). The aim of the present work was to investigate the behavioural parameters of these animals with open field, time-to-emerge and plus maze methods. Furthermore, we aimed at to examine the synaptic density in three regions of the CNS (the cortical pyramidal cell apical dendrites in layer 4; the molecular layer of the cerebellum and the main dendrites of the pyramidal cells of the hippocampal CA1 region) that are known to play a major role in motor and behavioural activity.

Materials and methods

Inbred strain of Wistar rats (16th generation) were used in these experiments. Animals (n=22 in each group) were selected for their voluntary ethanol consumption with a preference index (see SHULZ, 1987) larger than 0.7, and tested in the behavioural experiments described below (P animals). Control groups (n=25) were chosen from NP rats of the same inbred strain (preference index<0.20). All groups of animals were caged individually and kept under a 12–12h light-dark cycle. Food and water was available ad libitum. Temperature of the breeding room was 22–25 °C, the relative humidity about 50%.

a. Behavioural tests

1. *Open field test* (OFT; HALL, 1934)

An 8×8 square white painted 100×100×40 cm wooden test box was illuminated by a 150 W electric bulb from 150 cm above. A background noise was constantly applied which was about 20 dB strong. The experimental animals were placed individually in the middle of the box. The length of a session was 5 min and the experiments were repeated on 3 consecutive days. The parameters examined were the number of ambulations in outer and inner squares, the total ambulation activity in the first minute, the ambulation in the inner squares, rearing activity and latency of rearing, grooming activity and latency of grooming, the defecation rate and latency of defecation.

2. *Time-to-emerge test* (TTE; CRAWLEY and GOODWIN, 1980)

The testing equipment consisted of two communicating parts: a black painted dark chamber and a white painted indirectly illuminated one. The size of both chambers was 30×20×30 cm and they were connected with a guillotine door. On the first day of the experiments, each animal was placed 4 times into the dark chamber for 5 min to allow habituation to occur. On the second day, animals were placed into the dark compartment for 5 min, then the quillotine door was opened. TTE latencies were recorded when the animals entered the light compartment with all 4 paws.

3. *Plus maze test* (PMT; HANDLEY and MITHANI, 1984)

The test apparatus consisted of 4 arms (45×10 cm each); two were open while the other two were closed with a 9 cm high wall. The maze was elevated 80 cm from ground level. Each animal was tested in a 5 min session and the following parameters were recorded: latency to leave the centre of the maze, first choice of the open or closed arm, the number of entries onto and time spent on open arms.

b. Electron microscopic processing

Brain tissues from animals used in behavioural tests were processed for electron microscopy. Both the P and NP groups (6—6 specimens: 3—3 males and females) were perfused transcardially under Nembutal anaesthesia. First with 0.12 M phosphate buffer (PB) followed by a fixative solution (4% paraformaldehyde, 2.5% glutaraldehyde in 0.12 M PB at pH 7.4) for 20 min. After removing the brains from the skull, small tissue blocks were cut from the parietal cerebral cortex close to the midline, the hippocampal grey matter in the CA1 region and the lower vermis region of the cerebellar cortex. The tissue pieces were postfixed in the same fixative for 3 h, then washed in 0.12 M PB containing 7.5% sucrose. Buffered 1% OsO_4 was applied for 1 h, followed by dehydration through an ethanol series and propylene oxide. Block contrasting took place in 70% ethanol saturated with uranyl acetate. Tissue pieces were then embedded in Durcupan ACM resin. Semithin and ultrathin sections were cut with a Reichert Om U2 microtome. The ultrathin sections were counterstained with lead citrate, viewed and photographed in a Jeol 100B or Tesla BS 540 electron microscope. In the case of the cerebral cortex, the number of synapses/100 μm of pyramidal cell apical dendrite membrane in layer 4 was recorded and the same parameter was determined in the molecular layer of the hippocampus. In the cerebellum, the synapse density in the molecular layer was calculated.

c. Statistical evaluation

The results of both the behavioural tests and also the EM observations were processed by using the information statistics (KULBACK, 1978). For establishing significances, the F-test was used.

Results

The alcohol preference index was determined for all the groups as described by SCHULZ (1987). The preference index of the female P rats was 0.83 ± 0.11, while that of the NP rats was 0.15 ± 0.13. It is interesting to note, that this index was smaller among the male P animals (0.6 ± 0.12), while in case of the NP animals it was 0.16 ± 0.07.

In the OFT, the males (Fig. 1) of the P strain had significantly higher inner ambulation rate; more wall rearings and groomings with shorter latency to these parameters were observed on the first day. The defecation rate of the P males was lower, than that of the NPs on all the days, and the latency of defecation was higher in P male animals. The female P rats (Fig. 2) showed much higher motility than the members of the NP group. There was a higher rate of both the total ambulation and the inner ambulation on the first day. The P females showed more rearing and grooming, and the latency of these parameters was mostly shorter than that of the NP females. The defecation rate of the P animals was also lower, while its latency was significantly longer on the first day only.

In the TTE test there was a significant difference in the latency of leaving the dark box both by the males and females (Fig. 3). The female P group showed an extremely short latency, while in the male P group the same parameter was substantially higher, than that of the NP males (Fig. 3).

In the PMT, the males differed in the latency of leaving the centre, which was prolonged in the NP group; in their entries to the open arm, which was highest for the P males. The time spent there was much less than that of the NP group. The defecation rate of the P males was higher (Fig. 4).

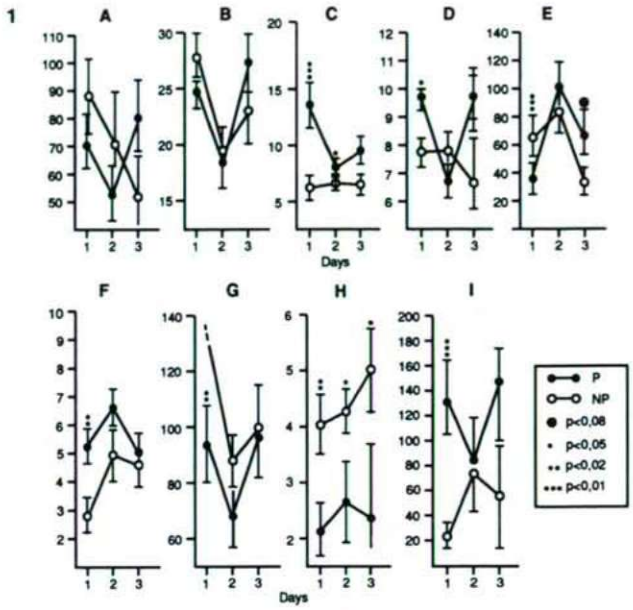
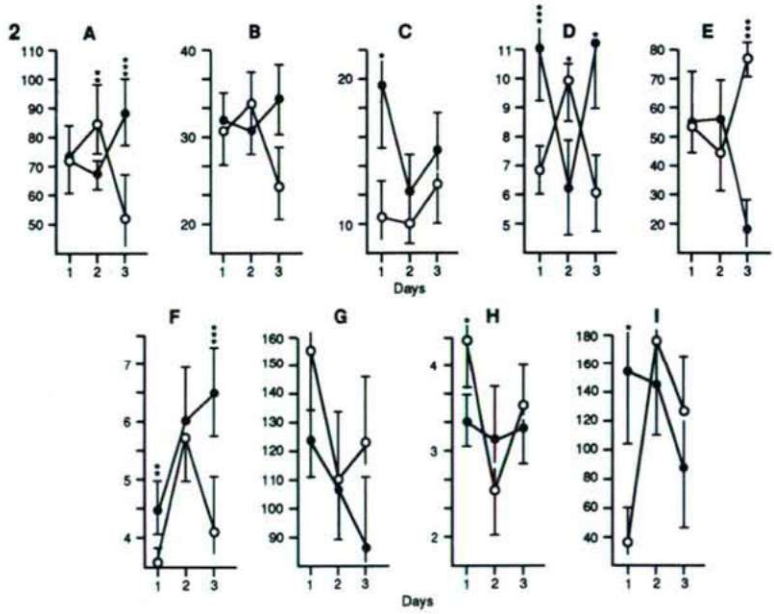


Fig. 1. The results of the open-field test. The filled circles represent the P (preferring), while the empty circles the NP (non-preferring) males. A: total ambulations; B: ambulations in the first min; C: ambulations in the inner part of the open-field; D: wall rearing; E: latency of rearing; F: grooming; G: latency of grooming; H: defecation rate; I: latency of defecation. $p<0.08$; *$p<0.05$; **$p<0.02$; ***$p<0.01$. The test days are indicated on the abscissa, while the time in secs on the ordinate. Conventions also apply to the Figures 2—4.



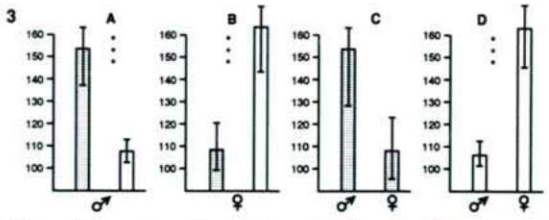


Fig 3. The TTE test latencies compared to each other in two different ways. A and B show the results of P and NP males and females, while in the C, and D graphs the two sexes of the same strain.

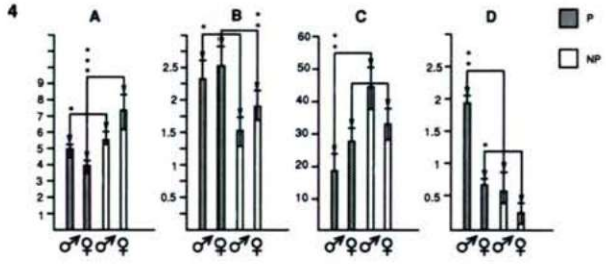


Fig. 4. The results of the plus maze test in bargraphs. A: Latency of leaving the centre; B: first choice; C: the number of the entries to the open arm; D: time spent in the open arm.

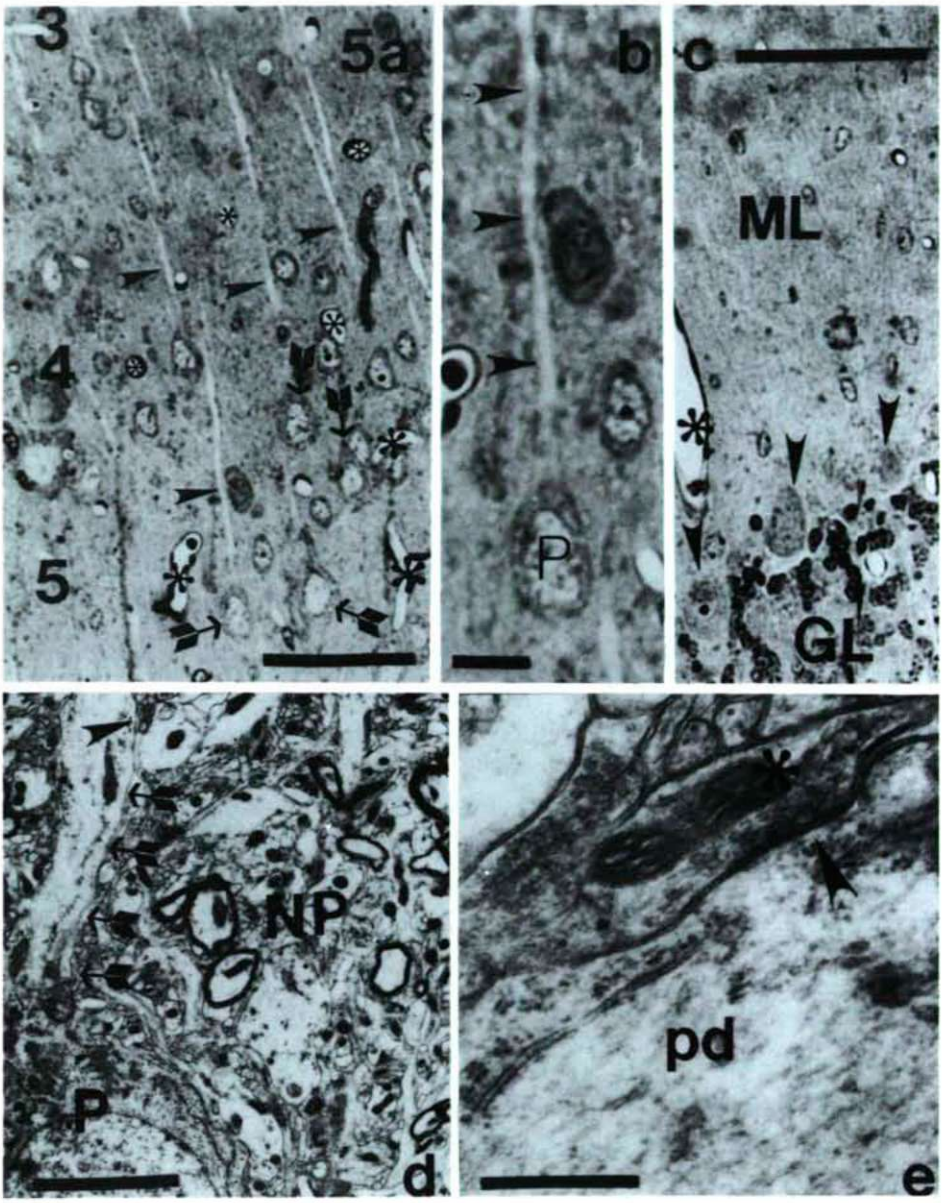
There was no difference in the entries to the open arm between the P and NP females, but all of the other parameters were similar to that of the males (Fig. 4).

The results of the synapse counting are summarised in Table I. Some of the sample areas are shown in Fig. 5. There was a significantly lower synapse number in the P strain on the pyramidal cell apical dendrites in layer 4 of the cerebral cortex, while in the molecular layer of the cerebellar cortex the density of synapses was higher in the P group. The two strains did not differ significantly from each other in number of synapses converging to the apical dendrites of the hippocampal pyramidal cells in the molecular layer.

Discussion

The breeding of our P strain of rats has now reached the 16th generation. During this time, it was possible to follow the diverging and common elements in the behaviour of P rats compared to the control group, with continuous screening. The changes clearly reflect the differences in the emotional state of P and NP rats, which may be related to alterations within the CNS. Thus, in parallel to

Fig. 2. The open-field results of the female groups. The individual graphs A—I present the same parameters as in Fig. 1.



the behavioural experiments, we hoped to find ultrastructural alterations in important brain centres such as cerebral cortex, hippocampus and cerebellar cortex.

It is necessary to make a clear distinction between alcohol dependence and preference. Physical alcohol dependence could not be developed during our

Fig. 5. The sample areas for electron microscopy from the P animals. A: low power photograph of the cerebral cortex showing layers 3, 4 and 5, with pyramidal neurons (arrows) in layer 5 and their apical dendrites in layer 4 (arrowheads). Asterisks: capillaries. Scale bar: 15 μm. B: The initial part of a pyramidal cell (P) dendrite (asterisks). Scale bar: 15 μm. C: Cerebellar cortex. ML: molecular layer, GL: Granule cell layer, arrowheads: Purkinje cell, asterisks: capillary. Scale bar: 10 μm. D: Low power electron micrograph of a hippocampal pyramidal neuron (P). NP: neuropile, arrows: pyramidal cell dendrites, arrowhead: synaptic connection shown with higher magnification in E. Scale bar: 5 μm. E: Symmetric synaptic contact (arrowhead) to a pyramidal cell dendrite (d) from a clear-vesicle containing profile (asterisks). Scale bar: 100 nm.

breeding procedure, since the experimental animals received ethanol ad libitum 4 times only. The volume of the consumed ethanol and the low number of drinking trials is not enough for the emergence of an alcohol dependence (see SHULZ, 1987).

Among the OFT parameters the ambulations and the rearing are fear-motivated (HALL, 1934; SANTACANA et al., 1972; WALSH and CUMMINS, 1976). As presented in the Results, these parameters were at higher values in P rats in both male and female populations. The higher intensity of movements shows a higher level of fear, against the lower defecation levels with longer latency. At the same time, the high grooming activity of the P animals allows the speculation, that unusual environmental cues play important roles in the activation of the attention system of these animals.

In the TTE test, a conflict situation (i. e. dark preference versus curiosity) is employed, to examine the decision making and fear-overcoming process, when the animals have to leave the dark well known chamber to enter the light, not known part of the test box. Interestingly, only the female P rats left the dark box with a significantly shorter latency, while the results of the P and NP males were opposite. These results are important when considering the human analogy where the female alcohol—driven behaviour is even more uncontrolled than that of the males (MILLER et al., 1989; SCHMIDT et al., 1990).

A higher threshold of fear and a more uncontrolled behaviour of female rats can also be observed in the PMT test. Both the short latency of leaving the

Table 1. The number of synapses in the three brain areas, mean and standard deviation

	CORTEX No/100 μm membrane	CEREBELLUM No/100 μm^2	HIPPOCAMPUS No/100 μm membrane
P strain	9±4.1*	51±10.2*	14±3.6
NP strain	16±2.7	36±8.8	17±4.2

* — $p<0.025$ significant difference between the elements of the same column.

centre of the maze and the number of the entries to the open arms support this view.

Considering the results of the 3 behavioural tests we concluded that the P rats showed a modified behavioural pattern. The fear-motivated elements in the behaviour were far less dominant in test situations than in the NP rats. Also, they showed a substantially higher level of motor activity, which has already been shown in P rats in response to low dose ethanol treatment by others (WALLER et al., 1984).

The results obtained in the behavioural tests made it possible to consider, that both major limbic and motor structures might have been genetically affected during the forced drinking experience followed by the selection procedure. Previous findings have shown that chronic ethanol consumption reduces the number of dendritic spines on the pyramidal cells in the cerebral cortex of humans (FERRER et al., 1986) and in the hippocampal CA1 pyramidal cells in experimental animals (McMULLEN et al., 1984). Reduction of the Purkinje cell dendritic tree after chronic ethanol treatment has also been observed (PENTNEY, 1982). Our results cannot be directly compared to those of the above cited studies, since our experimental animals did not get enough alcohol to be considered as a chronic treatment. Furthermore, the tendency of the changes revealed by our experiments, suggests a kind of compensatory mechanism in different brain centres. While the hippocampal CA1 region remained relatively unchanged, the number of synapses on the cerebral pyramidal neurons dramatically decreased. At the same time, the cerebellar molecular layer seemed to be enriched in synapses. A possible explanation for this fact is, that in parallel with the lower density of synapses in certain associative centres, a higher density of them may be formed in certain motor regions like cerebellum. This may be partly in connection with the higher motoric activity of P rats (WALLER et al., 1984). However these speculations need further experimental corroboration.

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DISEASES IN THE LATE ANTIQUITY: PALEOPATHOLOGICAL INVESTIGATION OF TWO ANTHROPOLOGICAL SERIES FROM FRANCE (3RD TO 4TH CENTURIES A.D.)

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Abstract

Completing an international research program focussed on the study of activity-induced pathologies and other pathological conditions on human skeletal material, we have analyzed two osteo-archaeological samples. The skelton remains of both cemeteries come from the Late Antiquity Period in France (Provence; South-East of France).

The cases we have examined come from the necropolises of "La Roquebrussanne" (18 skeletons) and "Sollies Toucas" (15 skeletons).

We found traces of traumas, enthesopathies, hyperostotic diseases (DISH), periostitis, perinatal infection, dental pathologies and a number of localisations of osteoarthrosis. Beside other disease types, a case of a fully-developed ankylosing vertebral hyperostosis (La Roquebrussanne) and one of a serious polytraumatism (Sollies-Toucas) are presented. The relatively high level of traumas is noticeable.

The differential diagnosis was carried out by macroscopic morphological and radiological methods.

Key words: paleopathology, Late Antiquity, France.

Introduction

Provence, a historical and geograghical region in the South-East of France provides a very abundant archaeological and human skeletal material dating from the Late Antiquity (BRUN et al., 1985; BOYER et al., 1987; FEVRIER et al., 1989).

The results of the anthropological and paleopathological examination of some of these series, such as the series of Marseille (MAFART, 1980) Fréjus (BERATO et al., 1990; DUTOUR et al., 1991), or Costebelle (DUTOUR and BERATO, 1991; PÀLFI et al., 1992) have already been published.

The aim of the present research program is to define and diagnose the pathological conditions of two human populations of the above mentioned historical period: skeletal material coming from the necropolis of La Roquebrus-

sanne (4th to 5th centuries A.D.) and from one of Sollies-Toucas (4th to 5th centuries A.D.).

The survey is part of a research program entitled "Diseases, Activities and Environments of Ancient Populations in Central and Western Europe".

Materials and methods

The first skleleton series we examined belongs to La Roquebrussanne-cemetery, located at 35 kms to the North of Toulon. During a rescue-excavation executed by the archaeologists of the Archaeological Center of Var Region (C. A. V.) 19 graves were explored in 1981 (LEVEN, 1981). The skeletal remains of 18 individuals were dug out of those graves; most of them were fragmentary or in a mediocre state of preservation.

The second series comes from the rescue-excavation of Sollies-Toucas (situated at 20 kms of to the North-East of Toulon) which was executed in 1991. The excavations were directed by MICHEL PASQUALINI (PASQUALINI, 1991). The state of preservation of the 15 human skeletons is a little better than in the case mentioned above. The skeletal material is stored at the collection of the Archaeological Center of Var Region.

The aim of our work was to assess the pathological changes detected on the above mentioned skeletons. That assessment and the determination of sexes and ages at death were carried out by macroscopic morphological methods and taking the corresponding special literature into consideration. We had to use X-ray analysis to be able to identify the more difficult pathological cases.

Although we examined the 33 skeleton remains of the two series with the purpose of recognizing and identifying the alterations of pathological origin, it was evidently necessary to do a preliminary anthropological analysis.

The determination of sexes was made by means of methods used in phisical anthropology (FEREMBACH et al., 1986; MARTIN and KNUSSMANN, 1988). We determined the age at death of infantile or adolescent skeletons using the methods proposed by SCHINZ et al., (1952), STLOUKAL and HANAKOVÀ (1978) and UBELAKER (cit. MARTIN and KNUSSMANN, 1988). As far as the age determination of the adults is concerned, we did not carry out in practice the complex method proposed by the Workshop of European Anthropologists (1980). Though is seems to be fairly reliable, the analyses by MASSET (1982; 1986) have made doubtful the use of suture-closing in a correct age determination. The trabecular structure, frequently modified by osteoporosis (LAVAL-JEANTET and CAULIN, 1981), especially in female skeletons is not a clearly age-related characteristic either. So, based essentially on the criteria of the European Workshop, on the age-related changes at the pubic symphyses especially (NEMESKÉRI et al., 1960) and taking into consideration some other conditions (the calcification of cartilages, dental attrition, etc.), we carried out a more careful age estimation (DUTOUR, 1989). The sex and age group distributions of the two populations are presented in Table I.

Results and Discussion

1. *"La Roquebrussanne" series*

— Case Nr. 1: Grave Nr. 1; Male skeleton. Senile adult; mediocre state of preservation. The thoracic spine shows a right-side continuous bony overgrowth from T6 to T10. (Fig. 1). A compression fracture is seen on T11, too. The lateral X-ray of the same specimen (Fig. 2) presents the hyperostosic changes of the upper thoracic region as well. Enthesopathic osteophytes were observed in both the humerus, calcaneus, patella and the innominate bones.

Table I.: Sex and age group distribution of the two populations
La Roquebrussanne: R; Sollies-Toucas: S.

Sex Age at death	Male		Female		Un- determinable		Total		Total of the two series
	R	S	R	S	R	S	R	S	
Child	–	–	–	–	4,	2	4,	2	6
Adolescent	–	–	–	–	–	2	–	2	2
Young Adult	1	–	2,	2	–	–	3,	2	5
Mature Adult	2,	4	3,	1	–	–	5,	5	10
Senile Adult	1,	1	1,	2	–	–	2,	3	5
Undeterminable	2,	–	–	–	2,	1	4,	1	5
Total	6,	5	6,	5	6,	5	18,	15	33
Total of the two series	11		11		11		33		

Fig. 1 Ankylosing hyperostosis of the spine. Case Nr. 1: La Roquebrussanne, Grave Nr. 1, Male, Senile Adult.

Fig. 2 Lateral X-ray of the spine belonging to the Case Nr. 1.

These alterations correspond correctly to the criteria of a case of ankylosing hyperostosis of the spine (FORESTIER and ROTES-QUEROL, 1950), or of diffuse idiopathic skeletal hyperostosis (DISH) after RESNICK et al. (1975). Our case is very similar to the ones described in medical (LAGIER and BAUD, 1978; ARLET and MAZIERES, 1985) or paleopathological literature (ROGERS et al., 1985, 1987; KRAMAR et al., 1990).

Beside hyperostosic changes, the skeleton presents the signs of intervertebral osteochondrosis (from C4 to C6) and the osteoarthrosis of the posterior apophyseal joints (from C3 to C5). As far as the correlation of the two processes is concerned, there are, in our opinion, two independent diseases: hyperostosis and degenerative spinal disease, both of them being predominant in elderly age (LAGIER, 1982).

– Case Nr. 2: Grave Nr. 4; Male skeleton. Senile adult; mediocre state of preservation. There are signs of spinal osteophytosis from L1 to L4.

Osteoarthrosis is present on the first right metatarsophalangeal joint. It is a relatively frequent consequence of an instep-depression or a primary hallux valgus (CHAOUAT, 1970).

There is a healed spiral fracture of the left tibial diaphysis (Fig. 3). Remodelling after trauma and healing by callus formation can be seen. This type of

Fig. 3 Healed spiral fracture of the left tibia.
Case Nr. 2: La Roquebrussanne, Grave Nr. 4, Male, Senile Adult.

fracture necessitates a violent trauma and a long-lasting healing (ORENGO and TAYON, 1980). A healed fracture can be seen on the right clavicle, too. Although it concerns the most frequent traumatic lesion of the skeleton (COSTAGLIOLA, 1976), the double fracture suggests a serious accident of our specimen.

— Case Nr. 3: Grave Nr. 13; Female skeleton. Mature adult; fragmentary state of preservation. Some signs of osteoarthrosis can be seen on the joint surfaces of the first and third proximal interphalangeal joints on the left, and on the second right distal interphalangeal joint. The first ones correspond to a Bouchard's arthrosis, the second one to a Heberden's arthrosis (GÖMÖR and BÀLINT, 1989).

— Case Nr. 4: Grave Nr. 19; Male skeleton. Adult, age undeterminable; fragmentary state of preservation.

There are signs of spinal osteophytosis of the lumbar spine, from L1 to L4.

2. *"Sollies-Toucas" series*

— Case Nr. 5: Grave Nr. 1; Female skeleton. Senile adult; fragmentary state of preservation. Some signs of osteoarthrosis can be detected on the left side temporomandibular joint (TMJ). The right side is not present. Our specimen's age and dental attrition of a high degree are connected with the degenerative disease, while the dental status, especially the dental attrition (HODGES, 1991), ante-mortem tooth loss and occlusal malfunction: the "TMJ disfunction syndrome" has a significant association with the TMJ osteoarthrosis.

— Case Nr. 6: Grave Nr. 2; Male skeleton. Mature adult, mediocre state of preservation. There is a unilateral spondylolysis of L4 (right side). The signs of a right-side spinal osteophytosis from T10 to L3 can also be detected. Spondylolysis, described as a skeletal malformation (ORTNER and PUTSCHAR, 1981) or as a fatigue fracture (MERBS, 1983, 1989), is associated with the degenerative joint disease of the lumbar spine (BRIDGES, 1989). In our case we suggest that the static disorder of the lumbar spine caused by the unilateral spondylolysis can also be responsible of the osteophytic process.

— Case Nr. 7: Grave Nr. 4; Female skeleton. Young adult: mediocre state of preservation. Enamel hypoplasia is expressed in the form of hypoplasic transverse lines of the buccal crown surface of all the teeth except the molars.

Some signs of a healed fracture can be seen on the left fibula, a little above its distal end. We can also detect periostitic changes on the surface of the left-side fibular incisure and the enthesopathies of the interosseous ligament on the same tibia. The fracture of the fibula can be estimated as a primary factor of these alterations.

— Case Nr. 8: Grave Nr. 5; Male skeleton. Mature adult, mediocre state of preservation. There are signs of spinal osteophytosis on the thoracic spine, from T6 to T12, with ankylosis of T7 and T8.

Enamel hypoplasia is present on all of the teeth of the mandible and the upper canines. The right TMJ is characterized by an osteoarthrosis, probably associated with the left-side antemortem tooth loss of the mandible. Fig. 4 presents a dental caries and the traces of an abscess of the first lower right molar.

Fig. 4 Dental caries and abscess of the first lower right molar.
Case Nr. 8: Sollies-Toucas, Grave Nr. 5, Male, Mature Adult.

There are caries of the second upper right incisor and the first upper left premolar; and the first lower left premolar.

– Case Nr. 9: Grave Nr. 8; Female skeleton. Mature adult; fragmentary state of preservation. A caries associated periapical abscess in presented on the first upper left premolar.

– Case Nr. 10: Grave Nr. 9. Male skeleton. Mature adult; fragmentary state of preservation. Both the tibiae and the fibulae are characterized by an expansive periosteal new bone formation (Fig. 5). The patellas and the right ulna also present periosteal appositions. There is a great deal of florid periosteal new bone, particularly on the tibiae. The new bone has some coarse striations and pitting (Fig. 5) and in some cases it is rugose (Fig. 6) on the tibias. The third right metacarpal presents periostitis and an osteolytic lesion near its distal end. There are signs of hypervascularisation on the knees. We have detected some traces of a right-side tricipital enthesopathy and bilateral cribra orbitalia.

Although several pathological conditions may be associated with periosteal bone reactions, we can note that the skeletal pattern and the morphology of these lesions could refer to a treponemal infection (HACKETT, 1976; STIRLAND, 1991). Unfortunately, the very fragmentary state of preservation and the lack of several elements of the skeleton prevent us from establishing a precise diagnosis.

– Case Nr. 11: Grave Nr. 12; Incomplete skeleton of a newborn. Good state of preservation. It concerns the incomplete skeletal remains of a newborn (around 10 lunar mounth old after STLOUKAL and HANAKOVÀ, 1978). All the bones present for the analysis are covered by a periosteal new bone formation (Fig. 7). The reactive new bone has a thickened porous nature and gives a "hairy"-character to the bones.

As we cannot know the exact age of the child at the moment of its death, it

Fig. 5 Periosteal new bone formation on the left tibia.
Case Nr. 10: Sollies-Toucas, Grave Nr. 9, Male, Mature Adult.

Fig. 6 Rugose periosteal appositions on the right tibia belonging to the Case Nr. 10

is impossible to decide whether it concerns a perinatal or a fetal infection. Perinatal bacterial infections, causing periosteal bone formation and being frequently letal, are generally caused by *Staphylococcus aureus* or some *Streptococcus* species (SANTOS and HILL, 1982; BEGUE and ASTRUC, 1988). SHULTZ (1984) presented the similar pathological alterations of a prehistoric newborn skeleton, as a case of osteomyelitis. In the case of a congenital infectious disease, venereal treponematosis can provoke bilateral osteoperiostitis (NABARRO, 1954; DELAHAYE and BEZES, 1979). It cannot be excluded at all because a case of early congenital syphilis from the 4th century has already been reported from the region (PÀLFI et al., 1991).

– Case Nr. 12: Grave Nr. 14; Skeleton of sex undeterminable. Adult, undeterminable; fragmentary state of preservation.

It shows a bony fusion of the left distal tibia and fibia following the fracture of the last one. The simultaneous trauma of the tibia is probable but the material is too fragmentary so we could not make a diagnosis. There are signs of an osteoarthrosis of the left ankle, as a possible consequence of the trauma. The osteoarthrosis of the first left metatarsophalangeal joint is also detectable.

– Case Nr. 13: Grave Nr. 15; Male skeleton. Adult mature: good state of

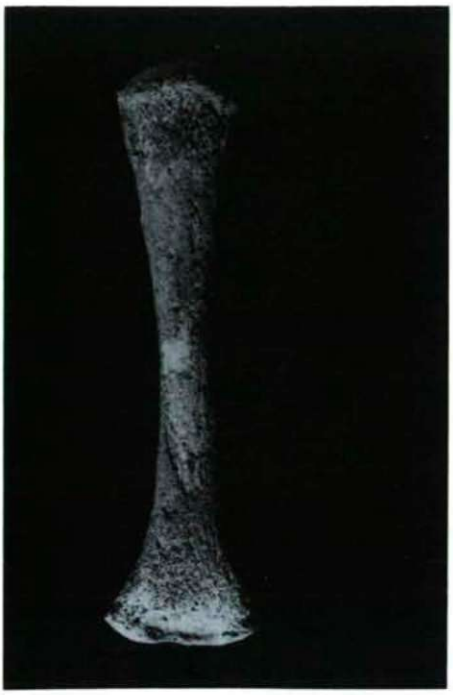

Fig. 7 Periostitis on the humerus of a newborn. Case Nr. 11: Sollies-Toucas, Grave Nr. 12, Newborn Child.

Fig. 8 Left and right humeri of the Case Nr. 13. The left humerus presents serious lesions.
Case Nr. 13: Sollies-Toucas, Grave Nr. 15, Male, Mature Adult.

Fig. 9 Osteoarthrosis of the glenoid fossa of the left scapula belonging to the Case Nr. 13.

preservation. This specimen presents several pathological alterations: Left humerus: it shows the shortened length and the destruction of the humeral head (Fig. 8). (The left humerus is 65 mms shorter than the right one.) The left shoulder joint is extremely destroyed by osteoarthrosis; the involvement of the glenoid fossa of the scapula is seen in Fig. 9. The X-ray analysis presented the serious osteoarthrosis of the left shoulder and an axial deformity of the humerus.

Right clavicle: there is a well healed fracture of the right clavicle.

Mandible: A healed fracture can be detected under the right mandibular condyle.

Ribs: The fragments of two ribs presenting signs of fractures were found.

The macroscopic characteristics of the left humerus reminded us of some skeletal dysplasias, especially of mucopolysaccharidosis (ORTNER and PUTSCHAR, 1981), but the skeletal displasias generelly involve numerous bones of the skeletons and the detected traumas suggest a traumatic origin of the process. The morphological and radiological pictures reveal the possibility of a double trauma: a diaphyseal fracture and a trauma of the humeral head. The destruction of the humeral head could be explained by a rupture of blood vessels following a fracture of the anatomical neck (OLIVIER, 1983), but it does not explain the shortening of the bone. The traumatic interruption of the blood supply in a growing bone can produce abnormal shortening of the bone (ZUJOVIC and CARLIOZ, 1979), so we must think of some traumatism during the growth. A very likely planation is that of a severe polytraumatism of enfance/adolescence: the fracture of the humeral diaphysis, the luxation and necrosis of the proximal epiphysis; probably simultaneous fractures of the ribs, the mandible and the clavicle. These traumatic deformations — causing much suffering and pain to this man for 30—40 years after the accident — provoked numerous consequences: the severe osteoarthrosis of the left shoulder, that of the right sternoclavicular and the right temporomandibular joints. The functional reduction of the left arm resulted in the atrophy of the bones of the left forearm.

Beside the alterations of traumatic origin, our specimen suffered from other diseases too. There is a complete block of the vertebrae C6 and C7 and a partial fusion of C4 and C5 (Fig. 10). As a typical consequence of a static disorder of the spine, a severe intervertebral osteochondrosis and the osteoarthrosis of the apophyseal joints are detected between C3 and C6.

Our specimen suffered from a hyperostosic disease, as well. There is a spinal osteophytosis from the T11 to L5. Some extraspinal enthesopathies (calcanei, innominate bones, patellae, femora) can also be detected.

— Case Nr. 14: Grave Nr. 16; Male skeleton. Senile adult, good state of preservation. There is a spondylolysis and spondylolisthesis of L5, which provoked degenerative joint diseases of L4 and L5 (intervertebral osteochondrosis and osteoarthrosis of the apophyseal joints). Spondylolysis is typically associated with higher levels of osteoarthrosis around the fifth lumbar vertebra (BRIDGES, 1989).

The cervical spine (from C2 to C7) presents intervertebral osteochondrosis.

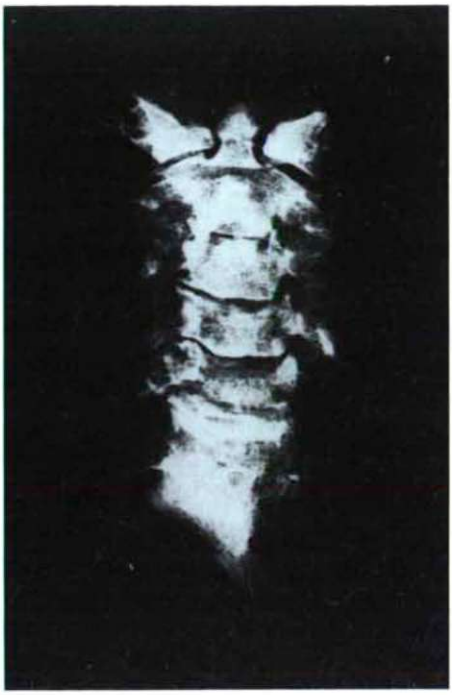

Fig. 10 X-ray picture of the cervical spine of the Case Nr. 13. Fusion of the vertebrae C6 and C7.

The osteoarthrosis of the hands and feet is also detectable: there is osteoarthrosis on the first left and second right metacarpophalangeal joints; and on the first left metatarsophalangeal joint.

Conclusions

The two populations on which the present study is based, lived approximately in the same historical period and under similar geographical conditions. Carrying out the paleoanthropoligical and paleopathological reconstruction of the skeletons we can gather a lot of information about their morphological characteristics and pathological conditions.

Within the limits due to the unequal preservation of the skeletons we conclude a relatively higher number of skeletal diseases in the "Sollies-Toucas" series. Although the restricted number of the skeletons in both samples excludes the precise analysis of the populations and their statistical comparison, the unevenness in remarkable: La Roquebrussanne: 4 pathological cases/18 skeletons; Sollies-Toucas: 10 pathological cases/15 skeletons.

As far as the different disease types are concerned, the predominance of

hyperostosic and degenerative joint diseases is not surprising: they are the most common skeletal pathological lesions since the dawn of civilization (DUTOUR, 1989; ROTHSCHILD, 1989). In several cases (the OA of the spine and the TMJ, or, the osteoarthrosis in large joints (shoulder, ankle)) the primary factors (malformartions, micro-, or macrotraumas) could be discovered.

Among 5 cases presenting traumas, the two polytraumatic cases are noticeable: Case Nr. 2: 2 fractures; Case Nr. 13: 5 fractures.

In the two cases with periostitis (Cases Nrs. 10 and 11) the occurrence of the infectious diseases is evident with an uncertain etiology. The possibility of treponemal infections cannot be excluded.

In order to be able to carry out a better comparison between the pathological conditions of Late-Antiquity populations and to reveal the epidemiological questions of the diseases, the importance of the further paleopathological examinations of this period is particularily emphasized.

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THE QUESTION MARKS OF THEORETICAL SYNTHESIS
(About the relation of theoretical biology and philosophy in Hungary)

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Abstract

Author writes about the condition of theoretical biology and its relation with philosophy. It is emphasised that the dominant ideology of the last decades, pretending a united thinking, has influenced the development of an integrative natural scientific thinking. The fact relevant tasks of education and research are derived from it.
Key words: theoretical thinking, theoretical biology, natural philosophy, Hungary.

Introduction

It is a great responsibility to draw a reliable picture of the development of theoretical thinking within biology because however much it is tried to fight against it, specialization within biology grows to huge dimensions and it is a great responsibility, because there are ones who show their results on their own.

The picture will be partial and think it is good if we draw the exterior bounds of message well in advance: the concrete questions of biology will not be mentioned, although some problems could be debated, but we are not so at home on this field as those who deal with this subject day by day and that is why we can hardly give clear solutions. The development of theoretical thinking and examination of its application remain our subject in Hungary and of course not all questions of detail can be discussed.

Theory takes a very contradictional place on the base of traditions in Hungarian biology.

In spite of this we have some high qualified theorists, theory has not already taken its place in the system of biology and theory is thought to be an "idle chat" which is a slight help with the solution of practical questions.

Although pioneers of theoretical biology derive historically from Hungary too (BAUER, 1967; BERTALANFFY, 1932, 1934) situation, which has developed in connection of theoretical thinking, can not be compared to those central role, what theory plays in European and North-American research (GARCIA, 1982; JACOB, 1970, MOZA, 1989; WADDINGTON, 1965).

One of its causes is our isolation, which can be experienced in connection of

theoretical thinking, otherwise the effect of the dominant ideology of the last decades can not be neglected. Several works were translated on the field, which clearly show the conceptual marks of philosphy (NOVINSZKIJ and PLATONOV, 1954; FROLOV, 1975) this formal theoretical, apparently organic union, had a paralysing effect on integrated thinking within specialized branches of science.

The anomalies of thinking of theoretical biology

The subject of biology, examining living nature, has significantly advanced, especially during the last decades. The significance of generalisation of accumulated knowledge as a result of research, its theoretical summary and apprehension overgrow the boundaries of biology.

It derives from the characteristics of biology that its subject adjoins from one side, the not living nature, on the other side society. So it is clear since its becoming a sufficient subject, results reached by biology, correlations and theories served unaviodable as a subject of philosophical-ideological analyses and valuation. The different systems — but especially those of their ambitions, were perfectness — handled the questions of origin of life, its essence, its concept, the development and determination of living nature, the origin of man or evolution and function of mind as essential problems.

In spite of these, we can state that the general theoretical evaluation of biology — although a lot of conceptions, with an ambition for approaching synthesis from different directions, have been published — still come up against a diffeculty (BRITTON, 1969; LAZCANO-ARANJO, 1985; CHANGEUX, 1985).

Some questions will be stressed and examined in detail among its reasons.

Variety of manifestation of life necessiated biology to convert to a system of branch sciences, examining the most different part fields. Certain scientific branches in biology have developed a particular method for examination, a lot of special independent institutions had been established and the consequence is an almost confused special literature. Such a situation has been established that it becomes more and more difficult for a researcher to follow the results of his own field of research or especially the ones of further branches of science and their registration.

The growing specialization and differentiation with the development of sciences and its manifestation within biology had a result that a great part of researchers had given up their earlier existed claim for the establishment of a comprehensive biological view and now they are satisfied with the modest purpose to work up the literature of direct research field and to place their results in the knowledge system of the given branch of science.

On the other hand the development of the last quarto of the century has deepened our knowledge in such an extent that some earlier "tight-cut" hypothesis should have been given up and a demand on creating of new synthesis appeared with a thunderous force. The situation is becoming more and more complicated through the evidence that the characteristics of many fields of research are becoming interdisciplinar. Biology has less and less the principle and

conceptual frame with the help its knowledge could be arranged satisfactorily and which could serve as a heuristic modell in the further phase of research.

Progress can be reached by the result of those efforts which release the outlined conflicts. The solution of this challenge can be born out of new synthesis, which can serve to create new thoughts and explanations.

Contemporal biology at home has such outstanding personages who — having become aware of the situation, instead of explaining the contradictional situation — made an attempt at establishing a modern synthesis. (GÁNTI, 1971; SZENTÁGOTHAI, 1979; CSÁNYI, 1976).

Now it became obvious that it was necessary within biology too, under descriptive — research method and "disunity" of analytical organism, that a reseracher should do an integrated activity and strive for a formulation of general conceptions or hypothesis based on scientific facts.

Not any synthesis of all knowledge is meant, made by one man — which is in fact impossible — but a new way of thinking. Its characteristic is that it starts from facts if it is necessary false or archaic interpretation frames are removed and facts are rearranged according to new concepts. New special scientific research is inspired through this and later it leads to a more comprehensive integration of the analysed facts.

Our knowledge is hold up by a widening horizon through such a natural way and it does not become — in ourselves important but — an incoherent mass of information. This work is made difficult that no theoretical biology on a solid basis has been accepted (disciplines analogous to theoretical physics or theoretical chemistry are meant here).

Our university education system lacks — except theoretical biology — such further synthetical subjects as social biology or socialanthropology.

Comprehensive works made by biologists have not taken their due place in scientific theory.

Philosophy seems to be too abstract for a lot of nature researchers to become a bearer of a theoretical synthesis required by them.

Scepticism for philosophy can grow to a total negation.

While there was an intensive activity with theoratical and philosophical questions of natural sciences in our country during the 60ies up to recent decades "natural philosophy" got into an "embarassing situation":

No disciplinar aspect could be realised, it could not assign its role in the summary structure of philosophy. Its student circle became irresolute because of the charge "a philosophy of no full value". The cooperation of scientists and philosophers could not be realised on the expected level and effectivity.

Its result was that the theoratical generalisations of biological knowledge and the methodological epistemological principles were all formulated out of philosophy.

In the formation of the situation, social-political and scientific history played of course a role. The example of the creation of a general nature picture as N. Hartmann's conception, because of the lack of know, became ineffective.

The "shy" curiosity of natural philosophy about the so called civil scientific

theory — originated from the critical position, that one of its tasks is to reveal their hidden idealistic face. As a result of this, real problems eliminated and also worth extrapolations.

This happened to cybernetics, sociobiology, ethology and several branches of anthropology.

Natural philosophy from dialectic materialism got into a constant hypostatical situatioin, it became terminologically stiff and was not able to any philosophical thinking, which developed together with science. There were of course exceptions, but till these days it did not become clear where natural philosophy its place had and what its role was within philosophy. Scientific researchers and philosophers lived together in a "mutual suspicion" instead of cooperation. It derived from this source that in the latest philosophical essays, exactness and clearness were shaded by gleaming formulations, but glemour is only a superficial phenomenon. In fact one can not get rid of the suspicion that terminology is often unnecessarily bombastic. On the other hand we must remember that a qualified psychologist is slightly in the situation that he could work himself into sciences in order to follow their results and what is more to consider it critically. Physical, chemical and biological topics are too many folded for it, they are complicated and progress is too quick.

Circumstances were a bit more favourable for natural scientists. Although they are also able to survey current research condititions on a special field — as it was described above — but their scientific grounding makes following of essential result of sciences easier. What is more, not only scientists had the claim to raise and discuss new results on a natural scientific base. (ERDEY-GRÙZ, 1965)

"Bridge-building" between biology and philosophy seems not to be an easy solvable work. Theoretical ambitions, published in home scientific reviews were not only qualified as "universal dilettantism", while philosophers often used the attribute "vulgar materialist of good intensions".

It is really a basic question what the scientific "deepness" is, where natural philosophy can reasonably "penetrate", on the other hand what the philosophical "highness" is, where theoratical biology should "raise"?

Understanding of the phenomena of living nature seems to be logical, during its explanation you should pay attention not to "absolve" philosophy in science because it can really lead to a positivist or vulgar materialist point of view.

Sticking in the level of general categories is so "dangerous" too — it must be so from the theoretical point of wiew — which can result an abstract apriorism.

Abstract products of scientific research, categories of theoretical science compared with empirical concepts are in a tighter relationship with philosophy. An often formulated viewpoint is that biological theory is not well developed enough, it falls behind the state and level of experimental research.

Interest in theoretical biology is growing therefore it is clear that philosophical research, which tries to reveal what way and how you can construate theoretical knowledge concerning the essence of living nature and its regularities become more and more important.

These investigations should clear which specifications they have gained what kind of nature modern biological knowledge became, thanks to those new methods which are nowadays not only experiments but applied in the sphere of theory. These investigations should reveal the regularities of logical and historical development of biology, the sources of development and its inner and outer factors.

Summarising the earlier sayings, the conclusion can be drawn that philosophy should arrange its relation with science again. Research work, which tried to reveal the inner logic of the development of natural sciences, plays an important role in it. Its aim is — among other things — to clarify the role of different scientific interpretations and mental trends and schools in the development and we meet a new problem: the backwardness of elaboration of biology in a scientific-historical way. (A good illustration of this situation is that there exists no education in scientific history of biology at the universities!)

Under the development of reserach of history of science, investigations of theory of science and methodology of science are considered to be important.

It is time to turn a severe attention to studies of different explanation types within biology and verification models and structures.

On the other hand — and it is perhaps more important — theoretical and methodological problems should be surveyed and as far as possible to answer those that certain biological scientific branches produce out of themselves during their independent development (e. g. humangenetics, sociobiology and molecular biology, etc.)

Theory of science — together with investigation of theory of science — seems to enjoy a unique prosperity in the future years and these topics can overtake the role, which the discipline "philosophical problems of natural science" within education and research as well during the past years played. When philosophical history based on classical texts becomes dominant in philosophical education, it stresses the probability of the above mentioned.

All these do not mean and can not mean that we should give up generalization of theoretical philosophy based on knowledge of natural sciences and analysis of philosophically seizeable aspects of scientific problems. It should be seen clearly — especially on the base of experience of the last decades that a theoretical work with an orientation for an objective world concept should exceed the reference circle of special scientific examination. An ontological interpretation is the internal demand of phylosophy, so it can not exist just as a mere illustrative function. Nature is not a "collection" of philosophy.

It is also a problem of history of philosophy how different, in principle divergent philosophies can integrate knowledge concerning nature? (Interpretation of the concept of „nature" should require an extra analysis.)

The problem is how "natural philosophy" can be inserted in the antropo-centric world concept of philosophy in the classical sense.

According to a socioontological viewpoint declared recently, knowledge concerning nature can not be the subject of philosophy, so any natural philosophy is impossible (VAJDA, 1967).

According to our point of view, general theoretical analysis concerning nature, can become the content elements of philosophy in so far as over exceeding empirism, in a wider sense, give a proper base about the place of man and his role and help with a higher development of old philosophical disputes.

So theoretical philosophy can not leave the problem out of consideration "man as an active subject" and it should distribute philosophical antropology on its own way.

It should clarify the natural particularities of man as a biological race (sex), his evoluationary-genetic abilities, individual mechanisms etc. i. e. all those physical-biological endowments which originally belong to man's bio-social totality. It should oppose historically exceeded false conceptions as the concept that man is solely the product of social developments and his natural biological endowments will dissolve in his socialness.

Some conclusions

In the consequence of philosophical research and education special theoretical sciences can be expected to gain a bigger significance and also theoretical biology.

Demand of interpretation, hypotesis and theoretical work will grow i. e. "a philosophical" moment will appear in special sciences. Some marks of integration will more and more emerge in the integration with the differentiation of sciences. During this process such special sciences will be established which would like to fill the role of philosophy — e. g. system theory will appear as much a science (KINDLER and KISS, 1969).

It is an important question in this situation how a more general theoretical synthesis can be established in one special science.

Two different integration ranges (they can be called a vertical and horizontal integration) do not preclude each other. At the same time theory of philosophy — especially epistemology — can grow in both of them. Biology can not do without a clear conceptual picture about itself.

In expert training of future researchers should be trained for a synthetic view and methodology and so that they should be able to coordinate the results of several scientific branches and they should turn from one research field to another that during the process they will not become unscientific.

All these will not preclude the so called traditional research fields and methods either. No scientific specialization will be argued, but such a specialization that starts work so, that it does make a survey of the whole field and it continues its activity that it does not fit its results in any kind of united frame.

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Short communication — Lettre

OBSERVATIONS OSTÉOARCHÉOLOGIQUES SUR LES SQUELETTES D'UNE SÉPULTURE GALLO-ROMAINE (GRÉOUX, FRANCE)

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Contexte archéologique et anthropologiqe

Suite à des labours dans un vignoble, la fouille d'urgence d'une tombe sous tuiles au lieu-dit Ravin Roubeau sur la commune de Gréoux dans les Alpes de Haute-Provence (90 km au nord-ouest de Toulon) a été effectuée par les auteurs en octobre 1992. Il s'agit d'un coffrage de tegulae rectangulaires (172×46×46 cm.), déposé dans une fosse creusée dans le substratum. La sépulture, en bon état malgré l'impact de la charrue, ne possédait pas de mobilier funéraire. Cette particularité ajoutée à son orientation (tête à l'ouest)et à son type de construction permet de la situer chronologiquement à partir du IVème siècle après J.-C.. Selon les témoignages des agriculteurs, il ne s'agit pas d'une tombe isolée, mais certainement d'une sépulture appartenant à une petite nécropole liée à une villa qui n'est pas encore localisée.

L'examen anthropologique in situ nous a relevé qu'il s'agissait d'une double inhumation : sur les pieds d'un sujet adulte les restes osseux déjà macérés d'un enfant, enterré probablement antérieurement ailleurs ont été superposés. L'analyse anthropologique en laboratoire nous a permis de préciser les données principales des deux squelettes:

1. *Squelette subadulte:* Il s'agit du squelette assez bien conservé d'un enfant d'environ 10 ans (dentition: 9-10 ans, longueur des diaphyses: 10 ans environ). Une malformation congénitale: spina bifida occulta au niveau des segments S1 à S4 et des variations anatomiques — foramen transversarium bipartita au niveau des vertèbres C5 et C6 ainsi que la présence des os wormiens lambdatiques — ont été découvertes.

2. *Squelete adulte:* Il s'agit du squelette en bon état de conservation d'un sujet adulte mature de 50 à 60 ans environ. La stature est relativement petite, 161±2,97 cm; le squelette est plutôt robuste d'après les indices de robustesse et les insertions musculaires. Le crâne (Fig. 1) est dolichocrâne (ICR: 73,82), orthocrâne et métriocrâne, sa capacité peut être estimée à 1524 cm^3 environ.
Le squelette présente de spina bifida occulta au niveau des segments L5 et et S1

Fig. 1: Le crâne du squelette adulte en vue latérale (x 0,3).

et une large série de variations anatomiques: foramen transversarium bipartita sur C6, bilatérale; présence d'os wormiens lambdatiques en nombre très élevé; anomalie transitionnelle thoraco-lombaire: aspect lombalisé de la vertèbre T12, athropie des dernières côtes; apophyses styloïdes asymétriques (celle du côté droit est soudée avec le cératohyal).

Examen paléopathologique

1. *Squelette subadulte:* La présence de cribra orbitalia bilatérale au niveau des voûtes orbitaires est à mentionner. Des phénomènes d'allure ostéolytiques peuvent être relevés sur quelques vertèbres lombaires et au niveau des genoux, mais le manque de lésions « positives » exclue leur différenciation de simples processus taphonomiques.

2. *Squelette adulte:* Plusieurs types d'altérations d'origine pathologique ont été relevés.

a) Traumatismes:

a1) L'humérus gauche présente une fracture médio-diaphysaire, consolidée par la production d'un cal exhubérant, sans glissement des fragments et sans raccourcissement de l'humérus.

a2) La 2ème côte droite présente une fracture consolidée.

a3) Lésions traumatiques de la région orbitaire: deux blessure rectilignes cicatrisées caractérise l'apophyse orbitaire externe et l'arcade orbitaire gauche (Fig. 2). Les directions des deux lésions sont nettements différentes (un angle de 15 à 20 degrés peut être estimé). Un fragment de l'arcade orbitaire a été décollé et nécrosé par suite des chocs. Une lésion arrondie est visible sur la voûte orbitaire frontale, sous la forme d'un orifice (9 mm×7 mm à bord lisse perforant la voûte et ouvrant le sinus frontal (Fig. 3). La quatrième altération pathologique de l'orbite gauche se manifeste par un enfoncement (18 mm×25 mm×9 mm) au niveau des os planum et lacrymal, accompagné par des aires nécrotiques.

Fig. 2: Lésions traumatiques de l'apophyse orbitaire externe et l'arcade orbitaire gauche (x 2,2).

b) Néoproduction anormale:
Une excroissance osseuse de dimensions 10×6×5 mm, évoquant un ostéome bénin est présent à la face interne de la mandibule, au dessous de la troisième molaire gauche, à la ligne mylo-hyoïdienne.
c) Pathologie dentaire et alvéolaire:
Une résorption alvéolaire de fort degré et la fréquence élevée de caries dentaires (8 des 20 dents sont atteintes (40%); le reste est perdu ante mortem), caractérisent la région alvéolaire. Deux abcès alvéolaires (associés aux caries) sont à mentionner.

Fig. 3: Perforation de la voûte orbitaire et destructions des os planum et lacrymal (x 1,2).

d) Arthropathies:
Des signes d'ostéochondrose vertébrale (discarthrose) sont relevés au niveau des segments C5 à C6 et L2 à L4; des discopathies sous forme d' empreintes de Schmorl évoluées sont visibles au niveau des vertèbres de T3 à L1. Une arthrose interapophysaiere unilatérale caractérise le segment C3 à C4. Les vertèbres T12 et L1 sont touchées par une arthrose inter-épineuse (syndrome de Baastrup).
e) Pathologie para-articulaire:
Les faces postérieures des astragales présentent une asymétrie très nette. Il s'agit d'un remaniement des tubercules postéro-externes: séparation de l'os trigonum à gauche, forme pseudarthrosique de l'os trigonum à droite (Fig. 4).

Conclusions

Bien que le volume de cette article ne nous permette pas de présenter une analyse anthropologique détaillée et la discussion complète des lésions mentionnées, l'importance de quelques observations particulièrement intéressantes nous oblige à consacrer un paragraphe à leur interprétation.

La présence de la même malformation congénitale et des deux variations anatomiques quasiment identiques suggèrent la possibilité d'un lien familial de ces deux individus. Ce lien de parenté pourrait expliquer l'exhumation du squelette de l'enfant et son enterrement avec le squelette adulte.

Les lésions pathologiques du sujet adulte ont plus de valeur diagnostique que celles de l'enfant. Dans le cas des fractures, la localisation suggère des origines différentes. la consolidation de la fracture de l'humérus est parfaite et sans rétrecissement fonctionnel du bras gauche d'après sa robustesse et des insertions musculaires très marquées du squelette de l'avant-bras.

Les lésions traumatiques de l'orbite gauche doivent être particulièrement soulignées. Les incisions linéaires sur l'arcade orbitaire gauche seraient dues aux chocs d'une lame mince (DASTUGUE et GERVAIS, 1992). La perforation de la voûte

Fig. 4: « Syndrome de la queue de l'astragale» . Séparation de l'os trigonum à gauche, forme pseudarthrosique à droite.

orbitaire peut être liée au même traumatisme fracturant également la voûte frontale. La dépression et la nécrose des os planum et lacrymal s'expliqueraient par leur fracture provoquée par un objet traversant la cavité orbitaire; dans ce cas la perte de la vue de l'oeil gauche est probable. Ni l'examen macroscopique, ni l'analyse radiologique ne nous ont permis de découvrir l'origine précise de ces lésions. Nous ne pouvons que conclure qu'il s'agit des conséquences d'une blessure (ou plusieurs) vraisemblablement provoquée par une arme et que l'invidu a survécu à traumatisme. Ce sont d'ailleurs ces sinus frontaux très larges (et l'arcade sourcilière très dévéloppée) qui lui ont « sauvé la vie »: le traumatisme n'a pas pu perforer la bosse orbitaire.

Dans le groupe des arthropathies dégénératives du rachis, le syndrome de Baastrup suggère une hyperlordose (Simon, 1989), mais la vertèbre T12, dont on note l'aspect transitionnel, pourrait également avoir été à l'origine de ce syndrome. Les altérations observées sur les faces postérieures des astragales révèlent un syndrome exostosant postérieur ou « syndrome de la queue de l'astragale » (Danowski et Chanussot, 1991). Ce syndrome d'hypersollicitation postérieure est lié à l'impact répété des tubercules postérieurs de l'astragale contre le rebord postérieur du tibia lors des flexions plantaires forcées. Ces lésions, comme les impacts d'autres microtraumatismes sur le squelette, encore peu reconnues par la paléopathologie peuvent nous livrer certaines informations fonctionelles (Dutour, 1992). Aujourd'hui, ce syndrome est reconnu comme microtraumatisme sportif, fréquent entre autres chez les coureurs (Claustre, 1987); chez notre sujet provenant de l'Antiquité Tardive le rôle de la course aurait pu également jouer un rôle important dans l'étiopathogénie des lésions observées.

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Short communication

PRESENCE OF ORCHIS TIMBALII IN ZSOMBÓ TERRITORY

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Orchis timbalii VELEN. 1882 is the hybrid species of *O. coriophora* and *O. laxiflora* subsp. *palustris*. Although the areas of the two taxa overlap large common territories in Hungary (cf. BORSOS, 1962; 1964), only some literary data are available on the presence and habitats of *O. timbalii* [at Kiskőrös (BOROS, 1923) and at Mórahalom (CSONGOR, 1992)]. BORSOS (1962, 1964) refers to some other data at Sári presented by PÉNZES and PRISZTER and by HORÁNSZKY at Székesfehérvár. SOÓ (1928) refers only to BOROS's date. Some other data are available from the personal communication with R. VIDÉKI: A small permanent population of *O. timbalii* can be found near the town of Cegléd, recent sporadical presences of the plant were recognized from sites that have been mentioned above. We are in lack of not only the floristical data but also the descriptions of cenological and environmental conditions.

In 1992 only one individual of *O. timbalii* was found in Zsombó territory (cf. BODROGKÖZY, 1974; CSONGOR, 1957). The plant flowered from 20 May to 7 June. It was 25 cm tall, the inflorescence had cc. 7 cm lenght. The number of the flowers was 14, mature fruit has not developed. The morphological features of the flowers can be studied in Figs. 1–3 of Plate I (Plate I).

The plant was found in a disturbed *Agrostio-Caricetum distantis* community, where the *Festuca pseudovina* formed a subassociation. Some *Molinion* species also occured in the studied vegetation spot. The list of species and their relative cover values (in percentages) are presented in the followings: *Agrostis stolonifera* 16, *Carex distans* 7, *Rhinanthus serotinus* 2, *Polygala comosa* 1, *Linum perenne* 1, *Plantago maritima* 3, *Silene vulgaris* 1, *Tetragonolobus maritimus* 1, *Plantago lanceolata* 1, *Lotus corniculatus* 1, *Achillea asplenifolia* 7, *Cerastium semidecandrum* 1, *Orchis coriophora* 1, *Orchis laxiflora* subsp. *palustris* 1, *Cichorium intybus* 1, *Euphrasia stricta* subsp. *suecica* 1, *Knautia arvensis* 2, *Festuca pseudovina* 25, *Poa angustifolia* 7, *Cynodon dactylon* 3, *Leontodon hispidus* 7, *Molinia coerulea* 1, *Serratula tinctoria* 1, *Ononis spinosa* 2, *Agropyron repens* 1. The total cover of vegetation is about 95%.

The soil of the habitat can be regarded as solonetzic meadow soil, which has moderately alkaline chemical reaction in its surfacial layer, the pH value slowly increases with the depth from 8.21 to 8.76 (the latest date regards to a soil sample from 50–60 cm depth). The thickness of soil samples is 10 cm. The calcium-carbonate content is between 32 and 38% in the soil samples. The surfacial soil layer contains 5.64% organic matter. The deeper layers have the

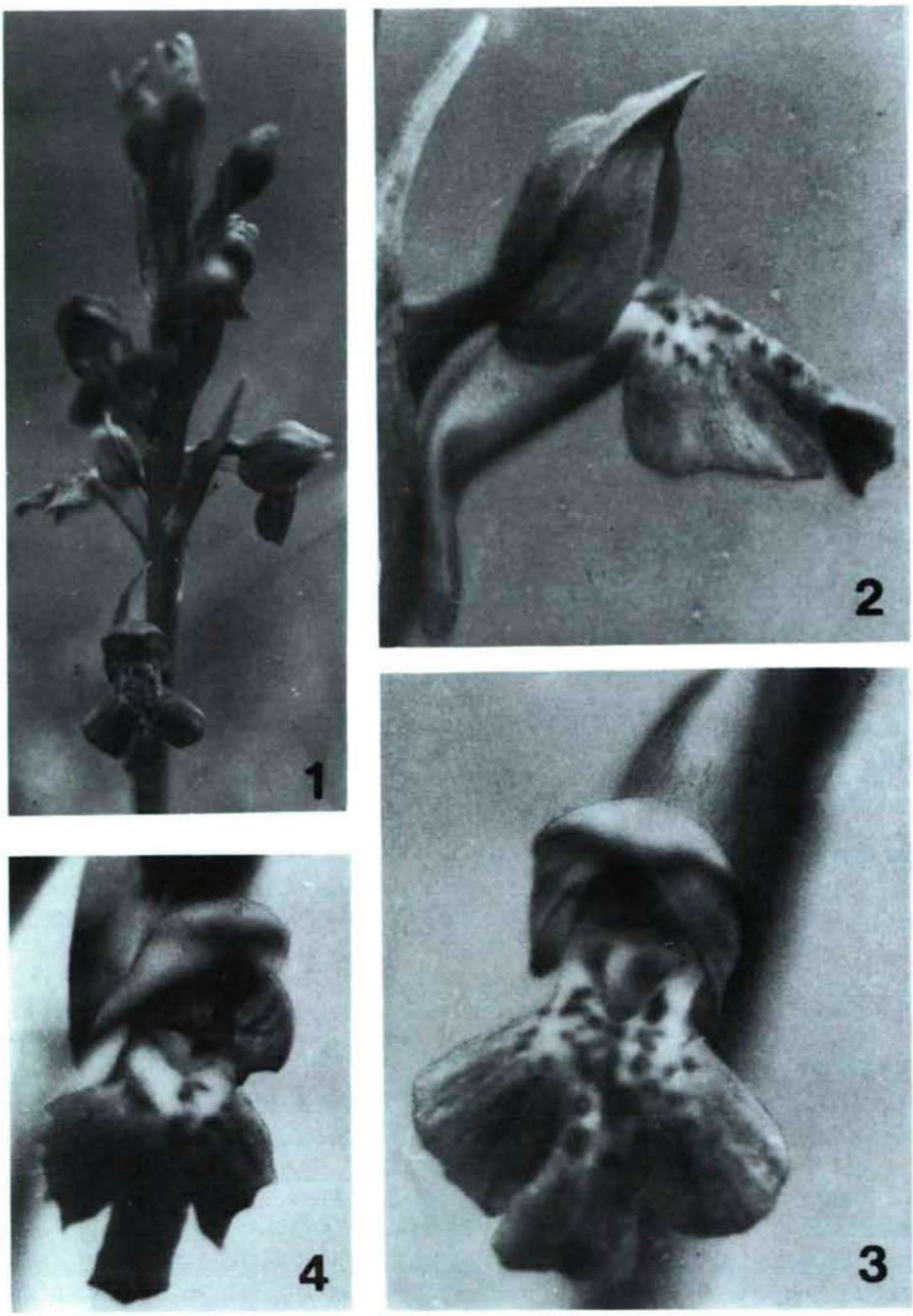

Plate I. Figs. 1—3: *Orchis timbalii*, Fig. 4: *O. coriophora*. The lateral sepals and petals as well as the dorsal sepal (cf. BELL, 1991) form a keel-like structure in both cases. In the Fig. 2 the spure was removed from the natural position.

following organic matter contents: 4.99, 2.36, 2.75, 1.04 and 0.88. The hygroscopic humidity (hy_1) — mainly due to the decrease of the organic matter content — decreases from 4.08 to 1.02%.

At the time of flowering of *O. timbalii*, there were cc. 1.500 individuals of *O. coriophora* and cc. 500 *O. laxiflora* subsp. *palustris* in flowers. The very low ratio of their hybrid species refers to the lack of their common pollinators. So the natural presence of *O. timbalii* may be a very accidental event.

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Thesis of dissertation for candidate degree

THERMAL STABILITY OF IMMOBILIZED ENZYMES, AND SOME PRACTICAL APPLICATIONS

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Introduction

As a result of the development and application of upto-date biotechnological methods during the past twenty years, together with utilization of the possibilities available in the fermentation industry, new prospects have emerged for the fermentation industry, new prospects have emerged for the preparation of numerous organic compounds. This has led to a "biological explosion"; it can be ascribed to the crisis situations facing mankind as a consequence of the rapid growth in the world's population and of the industrial revolution: food and energy crises, environmental catastrophes and to a certain extent the shortage of raw materials. Biotechnology is currently seeking a solution to many of these problems, which are at present impeding scientific and technical development.

Enzyme technology is one of the most intensively developing branches of biotechnology; it is closely connected with bioreactor planning and gene manipulation methods. Its aim is enzymatic biotransformation, involving the preparation of organic compounds, in one at most only a few steps. The main advantages of enzymatic reactions is their high degree of specificity and the mild reaction conditions. However, there are obstacles to the practical application of enzymes in dissolved form: in many cases their stability is not satisfactory, and additionally they themselves remain as contamination in the reaction mixture at the end of the reaction they catalyse. These problems may be eliminated by the use of immobilized enzymes: following separation of the products, such enzymes may be used again and the transformation may be made continuous, which decreases the costs of enzyme utilization.

The possibility of the continuous use of immobilized enzymes is primarily governed by two conditions: the planning of a suitable bioreactor and the stability of the immobilized enzyme. The steric structure of enzymes is basically stabilized by the the intramolecular interactions determined by the primary structure (MOZHAEV and MARTINEK, 1984), further contributions being made in vivo by interactions with the cellular protein and nonprotein components. Numerous enzymes are rather unstable in isolated form, which limits their practical application. The stability may be increased by making the globular structure more rigid through covalent modification (TORCHILIN et al., 1978; GERMAIN et al., 1989) and through immobilization of enzyme isolated from a source with favourable thermal stability.

Enzyme molecules can not be regarded as a homogeneous population with completely uniform structure: the steric structures and amino acid compositions of the individual molecules may differ slightly (COLVIN, 1954). Consequently, the individual proteins in the heterogeneous molecules are inactivated at different rates in response to heat treatment, i. e. the kinetics of thermal inactivation is not of first order. KAWAMURA et al. (1981) developed a model to characterize the thermal denaturing of immobilized α-chymotrypsin, which was described as a large number of parallel, independent, first-order reactions. On this basis, MALHOTRA and SADANA (1987) and HENLEY and SADANA (1989) considered that the molecular and stability properties of the microheterogeneous enzyme population exhibit a continuous distribution, and they devised a graphical thermal inactivation model which can be fitted well to the experimentally determined thermal inactivation time curves. By this means it is possible to calculate the average value of the activation energy, and also the standard deviation in the activation energy, which characterizes the degree of microheterogeneity, i. e. the thermal stability of the enzyme.

The stability of immobilized enzymes in generally described on an empirical basis: too few data are available as concerns the theoretical basis for it to be stated which factors determine the stability. Our research group has prepared a number of immobilized enzymes for practical purposes, the stability playing the determining role. With this research background, therefore, I began a study of the factors influencing the thermal stability of enzymes. The aims related to the following four areas:

1. A comparative investigation of the stability features of mammalian skeletal muscle aldolases, and selection of the enzyme with the highest thermal stability, which is most suitable for immobilization.
2. On the immobilization of enzymes with different molecular properties on supports with different physical and chemical properties, establishment of general regularities as concerns the catalytic and stability properties of the immobilized enzymes.
3. Numeral analysis of the complex thermal inactivation time curves, and hence study of the intra- and intermolecular changes influencing the conformational state of the immobilized enzymes.
4. Utilization of the immobilized enzyme reactors for analytical and preparative purposes.

Methods

Aldolase was isolated from pig and rabbit skeletal muscle, and triosephosphate isomerase from pig skeletal muscle, by literature methods.

The enzymes were immobilized via covalent bonding on Akrilex C—100 (polyacrylamide-based, with carboxylic functional groups), Akrilex AH—100 (with acid hydrazide functional groups), Akrilex P—100 (activated with p-benzoquinone) and Sepharose 4B (activated with cyanogen

bromide) supports, and also on inorganic, silica-based supports activated with glutaraldehyde or p-benzoquinone (Silochromes).

A study was made of the thermal stability of the enzymes immobilized on the supports with different physical and chemical properties. The complex thermal inactivation time curves, consisting of activation and inactivation stages, were analysed by two methods. The changes in K_m and V_{max} were measured in the activation stage of heat treatment. In the inactivation stage, a numerical method was used to determine the activation energy of inactivation, its rate constant, and the standard deviation in the activation energy, indicative of the microheterogeneity of the immobilized enzyme, based on the graphical model of HENLEY and SADANA (1989). The role played in the thermal stability by the secondary interactions between the enzyme and the support was investigated on the basis of the effects exerted on the thermal stability by the pH, ion and protein concentrations, and the substances binding specifically or non-specifically to the enzyme molecule.

On an Akrilex C—100 support activated with disubstitued carbodiimides with various structures and molecular dimensions, a study was made of how the presence of the activating agent on the surface of the support influences the coupling and orientation of the enzyme molecules.

The immobilized enzymes were used to solve various analytical and preparative tasks in a continuous, column or batch reactor. The enzymes immobilized on the inorganic, Silochrome supports were employed as bed. Fructose-1,6-diphosphate (FDP) was determined in the immobilized aldolase-triosephosphate (TPI)-glycerophosphate dehydrogenase (GDH) enzyme reactor, and glucose in the immobilized glucose oxidase (GOD)-peroxidase (POD) reactor, in a flow injection system. In the aldolase-triosephosphte isomerase reactor, dihydroxyacetone phosphate was prepared; glyceraldehyde-3-phophate was separated from the main product on Dowex 1×2 anion exchanger. In the glucose oxidase-catalase batch reactor, glucose was oxidized to gluconic acid.

Results

1. Pig and rabbit skeletal muscle aldolases differ substantially in their stability to heat and denaturing agents. The conformational stability of pig aldolase is higher than that of the rabbit enzyme. The two enzymes differ as concerns the pH optimum of the thermal stability and the thermal inactivation time curve. The difference in stability is justified by the difference in stability is justified by the difference in amino acid sequence, which exists despite the great structural homology of the enzymes of these two phylogenetically close species. On an industrial scale, aldolase is cheaper to isolate from pig skeletal muscle.

2. Surprisingly, when pig skeletal muscle aldolase undergoes covalent immobilization on the polyacrylamide-based support bearing carboxyl functional groups, the active lysyl side chain is not modified, and the immobilized enzyme has a high specific activity decrease. This is due to two reasons. Not only the side-chains containing the amino groups, but also side-chains of other amino acids participate in the covalent binding. Secondly, there are steric effects as a consequence of the different structures of the supports. These may lead to considerable differences in the steric structure of the molecule. Similarly to aldolase, TPI can be immobilized well on the extremely hydrophilic Acrilex.

3. From the aspect of immobilization of GOD, with the prosthetic group, the less hydrophilic environment, i. e. the inorganic support, is the more favourable. On coupling, the orientation of the enzyme is influenced fundamentally by the chemical properties of the functional groups of the matrix.

4. In response to covalent immobilization, the thermal stability of the enzymes increases, and the rate of thermal inactivation is lower than that of the dissolved enzyme. Accordingly, they are suitable for the study of changes in conformation of molecules. The thermal inactivation time curves are complex; in many cases they consist of an activation stage, followed by an inactivation stage. The changes in V_{max} and $K_{m\ app}$ in the activation stage point to the changes in the steric structure of the enzyme molecule. The immobilized enzyme molecule is in a metastable state, and its conformation is influenced by secondary non-covalent interactions. The secondary interactions may develop between enzyme and support, and between enzyme and enzyme, and they may be affected by variations in the immobilized protein concentration, the ion concentration and the pH.

5. For most of the immobilized enzymes we investigated, the inactivation stage can not be discribed by first-order kinetics. This indicates that the covalently bound molecules comprise a large number of populations with different thermal stability. For numeral characterization of the thermal staility of these microheterogeneous system, we took the model of HENLEY and SADANA (1989) as basis and devised a method for calculation of the activation energy. The activation energy values calculated for the inactivation stage provide a good characterization of the enzyme thermal stability, while the standard deviations of the activation energy indicate that the immobilized enzymes have a higher degree of heterogeneity than that of the dissolved enzyme. The greater the microheterogeneity of the immobilized enzyme molecules, the greater the thermal stability.

6. The effects of factors influencing the thermal stability of the immobilized enzymes, and the extents of these effects, can be studied on the basis of the $K_{m\ app}$ and V_{max} values measured in the activation stage of heat treatment, together with the activation energy of inactivation, its standard deviation, and the variation in the rate constant. The thermal stability of immobilized TPI is influenced by the pH, the ion concentration, and substances bindig specifically to the enzyme molecule. The presence of substrate does not alter the thermal stability appreciably, which indicates that protection of the active centre itself does not increase the stability. In contrast, the presence of phosphate ions leads to stabilization not only in the active centre, but also through coupling to the surface of the TPI molecule.

7. The conditions of enzyme immobilization influence the orientation of the enzyme molecule on covalent binding. The conformation and hence the thermal

stability of the enzymes immobilized on the Akrilex C—100 support are changed in different ways by the chemical and steric structures of the water-soluble disubstituted carbodiimides present as activators during immobilization. For carbodiimide derivatives with low bulk the amino groups of the enzyme become more accessible sterically on immobilization. Consequently, the immobilized enzymes have high thermal stability, and the standard deviation of the activation energy indicates a high degree of heterogeneity.

8. The immobilized enzymes were used for continuous analytical measurements in enzyme reactors, and in preparative operations. The concentrations of FDP and of glucose were determined in a flow injection system with an immobilized aldolase-TPI-GDH enzyme reactor and in a GOD-POD reactor, respectively. The latter system is suitable for the analysis of blood samples containing low glucose concentrations, such as the blood serum of fish in the resting state.

9. Dihydroxyacetone phosphate was prepared from FDP in a laboratory-scale, immobilized aldolase-TPI enzyme reactor. The reactor functioned with a maximum conversion of 70%, for 1 month, within any appreciable activity decrease.

10. Gluconic acid was prepared under regulated experimental conditions in an experimental GOD-catalase batch reactor. The productivity was 100—110 mmol/g enzyme/hour.

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ZUR ERINNERUNG AN PROF. DR. IMRE ANDRÁS LENGYEL (1934–1992)

Der Tod und die Vergänglichkeit erschüttern uns immer wieder von neuem, insbesondere aber immer dann, wenn wir von einem Kollegen, oder einem im wahrsten Sinne des Wortes hervorragenden Mann Abschied nehmen müssen.

PROF. DR. IMRE LENGYEL wurde am 11. September 1934 in Budapest als Sohn eines bekannten Juristen geboren. Seine Eltern verwandten große Aufmerksamkeit auf die Erziehung ihres Sohnes, der bereits als Kind in England seine erste Fremdsprache erlernte. In den dort verbrachten 10 Lebensjahren erwarb er die Sprachkenntnisse, die för sein späteres Leben von so ungeheur großem Nutzen waren.

Mit vierzehn Jahren trat er dann als Schüler in das Árpád-Gymnasium (Budapest) ein. In diesen Jahren lernte er die Traditionen seiner Heimat kennen und lieben. Nach Beendigung des Gymnasiums ließ er sich an der Medizinischen Fakultät der Budapester Universität inskribieren und erhielt dort 1958 das medizinische Doktordiplom.

Waren seine Interessen bischer doch sehr breit angelegt, so konzentrierte er sich nun auf die wissenschaftliche Ausbildung. In den Jahren von 1955–1957 betätigte er sich zuerst als Hilfsassistent und wurde so mit den Tätigkeiten in Lehre und Forschung vertraut. In dieser Zeit nahm er an zwei wissenschaftlichen Studentenwettbewerben teil und beteiligte sich bereits als Mitautor an dem von TIBOR DONÁTH herausgebenen Buch „Erklärung anatomischer Fachbegriffe".

Gleichzeitig beschäftigte er sich mit fluoreszenzmikroskopischen Untersuchungen.

Dezember 1957 nahm er eine Stellung als Praktikant im Anatomischen Institut an. Hier unterrichtete er zu Beginn seiner Hochschullehrerlaufbahn sowohl ungarische als auch Studenten aus Afrika; letztere in englischer Sprache. 1960 schrieb er für das Handbuch für medizinisch-technische Assistenten die Kapitel: Histologie, Pathohistologie, Histochemie und histochemische Techniken. In dieser Zeit begann er auch, sich mit der Histologie der Knochen sowie der Bio- und Histochemie zu beschäftigen.

Oktober 1960 nahm er seine Tätigkeit als klinischer Arzt (Assistenzarzt) in der Röntgenklinik der 1. Klinik für Innere Medizin an der Semmelweis-Universität Budapest auf, wo er bis zum 31. Dezember 1964 arbeitete. Über viele seiner überaus interessanten Arbeiten aus dieser Zeit hiel er Vorträge an den Universitäten von Mainz und Utrecht.

Januar 1965 war er praktischer Arzt für Allgemeinmedizin an einer Poliklinik im Stadtbezirk Pestszentimre, wo er bis aus Ende Mai 1977 blieb. Nach langer Krankheit setzte er dann seine Tätigkeit im Zentrallabor dieser Poliklinik fort und wurde 1978 Facharzt für Laboratoriumsdiagnostik.

1980 wechselte er dann an die Klinik für Gefäß- und Herzchirurgie der Semmelweis-Universität, wo er bis zu seinem Tod am 15. Juli 1992 das Diagnose-Labor leitete.

IMRE LENGYEL begann bereits in seiner frühen Studentenzeit mit Forschungsaufgaben, die er als junger Assistent z. T. sogar als Hobby erfolgreich fortsetzte und bis zu seinem Tod so intensiv weiterführte. Die Veränderungen der chemischen Zusammensetzung fossiler Knochen, Untersuchungen zu Geschlechtsunterschieden im Zitratgehalt bzw. der ABO Charakteristik in Knochen folgten. Angeregt worden war er durch Aufgabenstellungen des Budapester Anthropologen JÁNOS NEMESKÉRI. Dieser galt in Ungarn als der Begründer des paläoserologisch—paläoosteologischen Fachgebiet. Mit diesen Arbeiten begann und beendete DR. IMRE LENGYEL, zuerst als freischaffender wissenschaftlicher Mitarbeiter der Archäologischen Institute bei der Ungarischen Akademie der Wissenschaften seine Arbeit. Aus dieser Zeit und Schaffensperiode stammel seine engen Kontakte zu den Anthropologischen Sammlungen des Naturwissenschaftlichen Museums (Budapest) sowie zum Lehrstuhl für Anthropologie der Szegeder József-Attila-Universität. An letzterer Institution hielt er seit 1971 in insgesamt sieben Semestern Vorlesungen und leitete Praktika.

Seine intesive Publikationstätigkeit geht bis in das Jahr 1958 zurück. Von besonderer Bedeutung darf aus unserer Blickrichtung das Jahr 1963 angesehen werden, als er z. T. allein, zum anderen in Zusammenarbeit mit J. NEMESKÉRI eine Veröffentlichung über die theoretischen Grundlagen paläoserologischer und paläoosteologischer Untersuchungen vorlegte und dazu viele paraktische Befunde beisteuerte. Seine Arbeitsintensität war beachtlich, denn zwischen 1963 und 1983 erschienen von ihm 90 Publikationen in englischer, deutscher, spanischer, französischer und ungarischer Sprache.

Sein Untersuchungsmaterial der fossilen Knochen, einschließlich der mehr als 10000 Analysen dazu, umfaßte Reste aus dem frühen Neolithikum bis hin zum 19. Jahrhundert, eine Zeitspanne von mehr als 6000 Jahren, so sicherlich enzigartig im Weltmaßstabe. Seine enorme Leistung ist dabei mit Sicherheit seiner sehr guten Kenntnis der englischen, der deutschen, russischen und spanischen Sprache zu verdanken. Die durchaus glaubhafte Skelettresten nach anthropologischen Gesichtspunkten sowie deren archäologische Einordung war anhand seiner komplexen Methodologie möglich. Seine Ergebnisse halfen bei der Klärung ethnischer Fragen bestimmter Populationen, und deren vormals vorhandenen gesellschaftlichen Lebensformen ließen sich so besser rekonstruieren; so z. B. hinsichtlich der Unterschiede ethnischer Differenzen bei der Bestattung (geneologische Feststellungen).

Eine entscheidende Station in seinem Leben war das Jahr 1976, als er mit dem im Vorjahr erschienen Buch „Paleoserology" den Grundstein für seine Qualifikation zum „Kandidaten der biologischen Wissenschaften" legte. 1983 habilitierte er sich dann mit einer Arbeit unter dem Titel: „Populationsgenetische Ergebnisse aufgrund paläoserologischer Untersuchungen" auf dem Fachgebiet der Genetik.

Seine wissenschaftliche Arbeit bedeute ihm nicht nur tägliche geistige Auseinandersetzung und Vorbereitung, sondern auch ständiges Datensammeln und methodische Perfektion. IMRE LENGYEL lehrte als Hochschullehrer nich nur an der Semmelweis-Universität in Budapest, sondern auch an der Szegeder József-Attila-Universität und an der Eötvös-Loránd-Universität in Budapest. Auf Grund seiner vielseitigen Arbeiten erhielt er am 1. September 1979 eine Dozentur an der Eötvös-Loránd-Universität in Budapest und wurde zum Professor an der Semmelweis-Universität ernannt. In den letzen Jahren seines Lebens leitete er das Diagnose-Labor der Gefäß-und Herzchirurgischen Klinik in Budapest, wo er auch in der Arbeitsgruppe war, die in Ungarn die erste erfolgreiche Herztransplantation vorgenommen hatte.

IMRE LENGYEL war Mitglied mehrerer ungarischer sowie ausländischer Gesellschaften. So u. a. seit 1970 Mitglied in der Anthropologischen Komission der Ungarischen Akademie der Wissenschaften, seit 1980 der Klinisch-Biochemischen Komission. In letzter Zeit leitete er den Intelligenzklub, der Széchenyi-Gesellschaft.

Seit vielen Jahren hatte er sich nicht mehr politisch interessiert doch seit 1990 schloß er sich infolge der neuen gesellschaftlichen Verhältnisse in Ungarn der Christlich-Demokratischen Partei an und arbeitete aktiv in zwei speziellen Sektionen mit. Die „International Association of Human Genetists" und die „Paleopathological Association" hatten ihn mehrfach zum Mitglied ernannt. Er pflegte zahlreiche Kontakte zu ausländischen Kollegen, so u. a. mit dem Historischen Institut der Akademie der Wissenschaften in der ehemaligen DDR, der Humboldt-Universität zu Berlin, der Universität in Novi Sad (Jugoslawien), mit dem Institut für Anthropologie der Universitäten Utrecht und Pisa, mit dem Royal Museum in Toronto (Kanada), mit dem Archäologischen und Kunsthisto-

rischen Institut der Universität Villanova, mit dem Smithsonian Institut in Washington (U.S.A.), sowie zahlreichen anderen Institutionen. Viele Vorträge hatte er auf ausländischen Kongressen gehalten (1971, 1982 in Italien, 1980 in England, Frankreich, Holland, 1981 in Deutschland und Kanada). Die Wenner-Gren-Stiftung bezogen 1978 und 1980 das Centre National de la Recherche Scientifique bezogen seine Arbeiten in ihre Berichte ein.

Es ist außerordentlich schwer, sein Leben völlig darzustellen. Er war ein stiller, hilfsbereiter, und wie die Kollegen oft meinten, etwas bärenhaft wirkender Mensch, doch ein ehrlicher Freund und im wahrsten Sinne des Wortes, zutiefst intelligenter Mensch. Auch in schwierigen Zeiten blieb er zurückhaltend und lebte sein Leben 58 Jahre lang als Anhänger des protestanten Glaubens. Erst in den letzten 3 Jahren öffnete er sich auch hier und stellte seine Hilfe und Tatkraft zur Verfügung.

Nicht nur in der Wissenschaft, sondern auch im täglichen Leben wollte er schaffen. Seine gaplanten Vorhaben nahm er leider mit ins Grab. Er war oft wenig verschlossen, dennoch beispielhaft in seinen zwischenmenschlichen Beziehungen. Seine Eltern liebte er sehr. Mit dem Tod seiner Mutter zerriß die letzte Bindung zu seiner Familie; aber eben nur bis zu seinem Tod. Die Sehnsucht nach seinen Eltern hat nun auch ihn für immer ins Grab und zur letzten Ruhe gerufen.

Der Verfasser dieser Zeilen hatte oftmals die Gelegenheit, die beispielhaft in ungarischer oder englischer Sprache vorgetragenen Beiträge zu hören, die neben immer neuen sachlichen Informationen besonders durch ihren logischen Aufbau bestachen. Sowohl im Privatleben als auch in wissenschaftlichen Diskussionen äußerte er sich niemals lautstark, doch die Zuhörer lauschten seiner Argumentation.

Es ist noch immer unfaßlich, daß wegen eines fatalen technischen Fehlers, der zu einem Autounfall führte, ein so wertvolles Leben von uns genommen wurde; wo er doch mit Siherheit sowohl der Wissenschaft als auch uns im täglichen Leben noch so vieles hätte geben und vermittel können. Er hatte seine Ausbildung zum Arzt erfahren, war aber außerordentlich stark die Archäologie und der Anthropologie zugetan. Deshalb wird die ungarische Anthropologie ihm ein besonderes Andenken bewahren.

Gott mit Dir Imre! Dein so reiches Leben wird uns Vorbild sein.

GY. L. FARKAS

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PROF. (EMER.) DR. JÁNOS MEGYERI (1912–1991)

Betroffen nehmen wir zur Kenntnis, dass am 8. Dezember 1991 im Alter von 79 Jahren PROF. (emer.) DR. JÁNOS MEGYERI, Professor an der Pädagogischen Hochschule „Juhász Gyula" und an der Szent-Györgyi Albert Medizinischen Universität in Szeged verstorben ist.

JÁNOS MEGYERI wurde am 24. Juli 1919 in der Ortschaft Megyesbodzás im Bezirk Békés (Ungarn) geboren. Sein Vater war Landarbeiter, der infolge des 1. Weltkrieges schon früh Kriegsinvalide geworden war. Mit sehr grossen Anstrengungen half er seinem Sohn, dass dieser 1933 das Lehrer-Diplom erlang. Auf Grund der ausserordentlich schweren wirtschaftlichen Lage in dieser Zeit erhielt er trotz seines mit „sehr gut" abgelegten Diploms keine bezahlte Anstellung. So begann er mit einer Hilfslehrer-Tätigkeit in einem Szegeder Lehrlingsheim und wurde ab 1.9.1934 Erzieher in einem Internat für Lehrerbildung in dieser Stadt. Nebenbei arbeitete als Lehrer in der Praktikantenschule.

1936 schrieb er sich neben seiner Tätigkeit im Internat als Hörer an der Szegeder Pädagogischen Hoschschule für die Fächer Geographie und Biologie für Bürgerschulen ein. Hier erland er das Diplom im Juni 1940 als Lehrer für die Bürgerschule. Im Juni 1940 berief ihn PROF. DR. AMBRUS ÁBRAHÁM, damals Professor für Zoologie an der Pädagogischen Hochschule Szeged, als unbezahlten Assistenten an diesen Lehrstuhl. JÁNOS MEGYERI schrieb sich aber auch zur gleichen Zeit noch im sog. „Apponyi Kollegium", einer Weiterbildungseinrichtung mit Hochschulcharakter, ein, um hier die Lehrerlaubnis als Gymnasiallehrer zu erlangen. Zumindest nun musste er sich entscheiden, entweder die wissenschaftliche Laufbahn einzuschlagen oder Geld für die Sicherung seines Lebensunterhaltes zu verdienen. Am 5. Oktober 1940 nahm er eine bezahlte Stellte als Lehrer an der staatlichen Bürgerschule in Nagykanizsa an.

Aufgrund seiner erfolgreichen Tätigkeit als Lehrer Berief man ihn an die Szegeder Pädagogische Hochschule für Bürgerschullehrer als Fachlehrer an die Praktikantenschule dieser Lehrerausbildungseinrichtung. Neben seiner Tätigkeit als Lehrer arbeitete er regelmässig weiter am Lehrstuhl für Zoologie der Universität und beendete seine Studien am „Apponyi Kollegium" für Lehrerweiterbildung.

Am 10. Januar 1944 berief man ihn zum Militär und während seiner Soldatenzeit erhielt er im April dieses Jahres sein Diplom als Geographie- und Naturkunde-Lehrer an Lehrerbildungsinstituten. Am 12. Februar 1945 gelang er in Budapest in russische Kriegs-Gefangenschaft, aus der er 17. Juni 1947 nach Hause zurückkehrte und erneut als Lehrer an der Szegeder Praktikantenschule arbeitete.

Ab 1. Januar 1948 war er unbezahlter Assistent an der Naturwissenschaftlichen Fakultät der Szegeder Universität und promovierte am 30. April dieses Jahres zum Doktor für Philosophie mit einer Arbeit im Fach Zoologie und Anthropogeographie.

Am 3. Mai 1949 ernannte man ihn zum ordentlichen Lehrer an Lehrerausbil-

dungseinrichtungen und ab 2. Juli zum Lehrer am Lehrstuhl für Allgemeine Zoologie und Biologie. Ab 1. September war er unbezahlter Assistent am Zoologischen Lehrstuhl und am 15 März 1950 ernannte man ihn zum Oberassistenten. Im Januar 1952 wurde er stellvertretender Direktor der Naturwissenschaftlichen Bereiche an der Universität. Ab 1. September wurde er zum Hochschullehrer und Leiter das Zoologischen Lehrstuhls der seit dem Jahre 1975 mit dem Namen „Juhász-Gyula" verbundenen Pädagogischen Hochschule in Szeged ernannt, wo er bis zu seiner Emeritierung im Jahre 1975 ununterbrochen tätig war.

Am 5 März 1959 verteidigte er seine zweite Dissertation vor der Ungarischen Akademie der Wissenschaften zur Erlangung des Grades eines Kandidaten der biologischen Wissenschaft.

Für seine geleistete Arbeit wurde er mehrfach ausgezeichnet, so u. a. als „Bestarbeiter des Unterrichts" (1955), mit der „Dr. Entz-Géza-Urkunde" der Ungarischen Hydrobiologischen Gesellschaft (1964), mit dem „Orden der Arbeit" in Bronze (1966) und Silber (1973).

JÁNOS MEGYERI war in erster Linie Pädagoge mit einer ausgeprägten Persönlichkeitsstruktur. Sein Lebensweg vom Beginn als einfacher Lehre über die Stationen als Mittelschullehrer bis zum Ausscheiden als berufener Hochschullehrer war geprägt vom anspruchvollen Streben nach Erkenntnis.

Sein beruflicher Weg war nicht immer siegreich. In den ersten Jahren hatte er um seinen Lebensunterhalt zu kämpfen, dann zerstörte der 2. Weltkrieg seine Träume. Die zerrütteten Verhältnisse in der Nachkriegszeit und der frühe Tod seiner Frau und seines erst 20 Jahre alten Sonnes hatten ihn verbittert und liessen ihn oft schroff reagieren.

Er bemühte sich diese Lebensumstände zu meistern, indem er Ruhe und Ausgeglichenheit in der wissenschaftlichen Arbeit, in der Weitergabe seines Wissens und in der Prüfung der Kenntnisse seiner Hörer suchte. Er war einer stiller, geduldiger, aber fordernder Lehrer. Er war auf seine Weise hart, geradlinig; ein Lehrer, dessen Eigenschaften auch Widerstand hervorriefen, vor allem bei Leuten, die ihn nicht verstanden. Hier lag möglicherweise einer der Gründe dafür, dass er 1975 mit 63 Jahren und nach 23-jähriger Tätigkeit als Lehrstuhlleiter in einer Putsch-ähnlichen Aktion in den Ruhestand geschickt wurde. Diese persönliche Diskriminierung hatte ihn hart getroffen und fortan beschäftigte er sich nicht mehr mit seiner Wissenschaft (letzte Veröffentlichung 1975) und lebte von nun an zurückgezogen nur noch für seine Familie. Jene jedoch, die ihn kannten und weiterhin freundschaftlich zu ihm standen suchten seine anerkannte Urteilsfähigkeit und fanden auch weiterhin seine Hilfe und Unterstützung. Zu seinen Schülern, die ihm infolger fachlicher oder kollegialer Gemeinsamkeiten nahestanden, fühlte er sich engsten verbunden. Für sich nahm er fortwährend gewissenhafte und diziplinierte Arbeit in Anspruch, liebte Ordnung und Verantwortung.

Als konstruktiver Lehrer verfasste er eine Reihe von Lehrbriefen und auch Lehrbücher für seine Studenten.

In ihm lebte das Verlangen nach Erkenntnis der Natur. An der Seite seines wissenschaftlichen Lehrers PROF. A. ÁBRAHÁM eröffnete sich ihm die Möglichkeit zur Veröffentlichung von zehn wissenschaftlichen Arbeiten bzw. Büchern.

Seine wissenschaftliche Arbeit begann in den Jahren 1937–1938 mit der Veröffentlichung: „Die durch den Wind verwehten Pollen der Bäume Ungarns". Später folgten in erster Linie fachmethodische Artikel. Seit beginn der 50-er Jahre hatte er sich der Lebenswelt des Wassers zugewandt und begann mit der Hydrobiologie, einer Richtung, der er bis zum Ende seiner beruflichen Laufbahn treu blieb.

International war er nicht sehr bekannt, grosse Entdeckung hatte er nicht gemacht, dieses Glück war ihm nicht zuteilgeworden. Trotztdem deckte er in der vergleichenden Hydrofaunistik und Hydrobiologie, und seit Beginn der 70-er Jahre in der angewandten hydrobiologische Forschungen eine Reihe von Zusammenhängen auf. Er untersuchte die Schädlinge in den Reisfeldern Ostungarns, studierte die Tierwelt der Theiss und im „Weissen See" (Fehér-tó) bei Szeged, im Moor bei Bátorliget, in den Salzseen bei Bugac, in den Torfmooren bei Kelemér und Egerbakta, untersuchte die Lebewelt der Seen bei Szelidi und Baláta.

Staatliche Unterstützung erhielt er nur für Studienreise in die Tschechoslowakei (1955) und nach Albanien (1960). Er hielt mit seinem Lehrstuhl enge wissenschaftliche Verbindungen nach Österreich, Belgien, in die Tschechoslowakei, nach Frankreich, Holland, Kanada und Schweden. Er untersuchte vor allem die aus diesen Ländern zugestandten Planktonproben.

JÁNOS MEGYERI war mit dem wissenschaftlichen Leben seiner Umgebung eng verbunden. So war er z. B. Mitglied im Hydrobiologischen Kommittee bei der Biologischen Abteilung der Ungarischen Akademie der Wissenschaften, Mitglied der Kommission für Geschichte der Wissenschaft bei der Akademie, Mitglied in der Leitung der Hydrobiologischen Abteilung und Limnologischen Arbeitsgruppe der Szegeder Unterabteilung der Ungarischen Akademie der Wissenschaften. Er war einer der Themenverantwortlichen Szegeder Arbeitsgruppe für die Erforschung der Lebenswelt der Salzseen in den Nähe Szegeds im Rahmen des Internationalen Biologischen Programms (IBP). Er war Mitglied in der Leitung der ungarischen und der Szegeder-Sektion der Gesellschaft zur Verbreitung naturwissenschaftlicher Kenntnisse (TIT), stellvertretender Vorsitzender der Ungarischen (und der Szegeder Sektion) der Biologischen Gesellschaft. Er war weiterhin Vorsitzender der biologischen fachwissenschaftlichen Kommission beim ungarischen Unterrichtsministerium. In den Jahren 1959–1974 war er der Herausgeber des naturwissenschaftlichen Teils des Jahrbuchs der Szegeder Pädagogischen Hochschule „Juhász-Gyula", das später unter dem Namen "Wissenschaftliche Mitteilungen" erschien.

Mit dem Tod von PROFESSOR DR. JÁNOS MEGYERI endete der Lebensweg eines erfolgreichen Pädagogen und engagierten Wissenschaftslers, der gekennzeichnet war von grossem Fleiss und tiefer Verantwortung. Seine Schüler werden sein Lebenswerk in Ehren halten.

DR. GY. L. FARKAS

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BIBLIOGRAPHIE VON PROF. DR. JÁNOS MEGYERI

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CHRONICLE

Personalia

PROF. DR. E. HANUNOLD (Österreichisches Forschungszentrum, Institut für Landwirschaft, Seibersdorf, Austria) got the degree of a "Doctor Honoris Causa" at the József Attila University in Szeged.

DR. LÁSZLÓ ERDEI and DR. FERENC JOÓ (Biological Research Center of Hungarian Academy of Sciences, Szeged), DR. JÓZSEF FRANK (Research Institut of Cultivation, Szeged) have been appointed to honorary professor by the Senate of the József Attila University in Szeged.

PROF.DR. FERENC ZSOLDOS the Head of Department of Plant Physiology have been elected as a member of the Board of the European Nitrate and Ammonium Assimilation Groups.

ASS. PROF. DR. JÓZSEF TOLDI (Department of Comparative Physiology) have been charged with the affairs of the Department of Zoology for a definitive period.

The Bioecological Section of the Society of Hungarian Biochemists was founded in 1992 and ASS. PROF. DR. JÁNOS NEMCSÓK the Head of Department of Biochemistry was elected as the president of this section.

Awards

PROF. DR. ISTVÁN BENEDECZKY (Department of Zoology) was awarded the "Pedagogical Insignia" and ASS. PROF. DR. BÉLA MATKOVICS (Biological Isotope Laboratory) was awarded the "For the Hungarian University Education Medaillon" by the Hungarian Minister of Culture and Education.

Counsellor DR. GYULA DEZSŐ (Biological Section of Hungarian Academy of Sciences, Budapest) was awarded the "Bartucz Lajos Medallion" by the of József Attila University.

ASS. PROF. DR. SÁNDOR GULYÁS the Head of Department of Botany got the "Bessenyei György Medaillon" at the Teachers' Training College in Nyíregyháza.

PROF. DR. FERENC ZSOLDOS the Head of the Department of Plant Physiology got the divided prize of the Hungarian Academy of Sciences.

Scientific degree

ASS. PROF. DR. ATTILA BARANYI (Department of Comparative Physiology) took the degree of doctor in biological science with the dissertation "Biophysical and functional characterictics of neurocortical celltypes and their rule in associative processes at cellular level".

First Ass. Dr. Magda Gulyás (Department of Biochemistry) took the degree of candidate in biological science with the dissertation "Thermalstability of immobilized enzymes and their practical application".

Retiring

Prof. Dr. István Benedeczky (Department of Zoology) retired from 31. December 1992.

Scientific session

An International Symposium titled "Rules and Constraints of Community Assembly in Social Insects" was organized by the Department of Ecology from 24 to 29 August 1992 in Szeged.

Anniversary

Department of Comparative Physiology of the József Attila University was founded 25, and the Department of Microbiology of József Attila University was founded 20 years ago.

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